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ACRES DAVY McKEE ENGINEERING INC.

Toronto, Ontario

DESTRUCTION OF HUMIC ACIDS AND ORGANICS IN MINERAL DEPOSITS BY MICROBIAL ACTION STATE-OF-THE-ART REVIEW

FINAL REPORT

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BY:

ACRES DAVY McKEE ENGINEERING INC.

TORONTO, ONTARIO

March 25, 1985

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CANMET Scientific Authority: Mr. V. Sanmugasunderam

ADM Eng. Contract No. C1030.00

Arres Davy McKee

Engineers and Managers

March 25, 1985
C1030.00

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Dear Dr. Sanmugasunderam:

DSS File No. 15SQ.23440-4-9183
Contract Serial No. OSQ84-00208
ADM Eng. Contract No. C1030.00

Please find enclosed 11 copies of the final report entitled "Destruction of Humic Acids and Organics in Mineral Deposits by Microbial Action State-of-the-Art Review".

We have endeavored to clarify all the points raised during your review of the draft version of the report and we trust that the final version meets with your approval.

Should you have any questions, please do not hesitate to contact us. It has been a pleasure working with you on this interesting project.

Yours very truly,

David J. King

DJK:saf
Enclosure

D.J. King, P.Eng.
Project Manager

ARRES DAVY McKEE ENGINEERING INC.
Toronto, Ontario
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1 - INTRODUCTION

1 - INTRODUCTION

Acres Davy McKee Engineering Inc. (ADM Eng.) was awarded a contract by Department of Supply and Services for the study to review the State-of-the-Art for the destruction of humic acids and organics in mineral deposits by microbial action, DSS File No. 15SQ.23440-4-9183. Part of the work was subcontracted by ADM Eng. to the Ontario Research Foundation (ORF), Mississauga, Ontario.

We would like to thank the personnel at Alcan for their invaluable assistance during the performance of this study.

This report represents the final report for this project. Interim reports for Stages 1, 2 and 3 have already been submitted and are included as Sections 3 to 5 of this document.

1.1 - Objective

The overall objectives of this study are

(a) To collect and critically assess available information on the deleterious effects of humic substances on mineral processing operations. This will also include organic compounds formed from humic substances during the chemical processing of ores.

(b) To assess the technical and economic feasibility of utilizing micro-organisms to destroy or remove these troublesome organics from mineral processing operations.

(c) To document available information on candidate microbial processes to destroy humic substances and their by-products in mineral processing operations. If data are available, an outline will be included of the metabolic processes believed to be involved in the destruction of the organic materials.

2 - EXECUTIVE SUMMARY

2 - SOMMAIRE POUR L'EXÉCUTIF

La documentation pertinente a été dépouillée afin d'évaluer la faisabilité de la destruction des substances humeuses et des composés organiques présents dans les gisements de minerai. Ces recherches ont en outre été complétées par les observations recueillies au cours de discussions avec du personnel de l'industrie minière.

Les recherches et discussions démontrent que la présence de ces substances affecte plusieurs procédés de transformation (séparation des solides et des liquides, extraction de solvants, échange ionique, flottation); les problèmes rencontrés étant considérés saisonniers, excepté par les industries de l'uranium et de l'aluminium. Or, l'incidence de ces difficultés sur cette dernière a une portée incalculable sur l'ensemble de l'industrie minière canadienne.

L'industrie de l'aluminium souffre en particulier de la dégradation/désagrégation des substances humeuses et composés organiques durant l'application du procédé Bayer. La cristallisation de l'oxalate de sodium affecte la séparation des solides et des liquides et accroît la consommation d'hydrate de soude. Divers procédés de retrait des produits de dégradation/désagrégation d'un tel liquide ont été soumis au banc d'essai ou fait l'objet d'expériences pilotes, afin de solutionner ce problème, mais leur viabilité technique et économique n'a pas encore été évaluée.

La documentation dépouillée nous indique que la destruction biologique des substances humeuses et composés organiques dans la lessive Bayer pourrait s'avérer impossible, de par le degré élevé d'alcalinité. Par conséquent, une solution plus plausible consisterait à mettre au point un procédé de prétraitement du minerai avant l'étape du lessivage. Les diverses opérations résultant de l'utilisation de ce procédé ont été conceptualisées sur diagramme. Aucun microorganisme existant, qui aurait pu servir à la transformation de kérogènes et d'acides humiques, de poids moléculaire élevé, n'a été identifié. Cependant, d'autres microorganismes, qui ceux-là permettent une dégradation/désagrégation substantielle des acides fulviques et humiques de faible poids moléculaire que l'on retrouve dans les sols. Il pourrait s'avérer possible d'utiliser ces organismes pour prétraiter le minerai de bauxite.

2 - EXECUTIVE SUMMARY

A literature search to assess the feasibility of destroying humic substances and organics in mineral deposits by microbial action has been conducted. Discussions on this subject with personnel in the mining industry supplemented the literature search.

The literature search and personal contacts have shown that problems due to humics and organics occur in several unit processes (liquid-solid separation, solvent extraction, ion exchange, flotation) and are generally considered seasonal, except for the aluminum and uranium industries. The problem in the aluminum industry has greatest significance for the Canadian mining industry.

The aluminum industry encounters problems due to the degradation of humics and organics while processing the Bayer liquor. One of the degradation products is oxalic acid and the crystallization of sodium oxalate causes liquid solid separation problems and increased consumption of sodium hydroxide. Various alternative processes for the removal of the degradation products from such a liquid have been tested on bench and pilot scale, but their technical and economical viability have not been assessed.

Based upon the literature search, it is believed that the biological destruction of humics and organics in the Bayer liquor may not be possible due to the highly alkaline conditions. Consequently, a more likely solution is to develop a process to pretreat the ore prior to leaching. A conceptual flowsheet has been produced for this application. No existing candidate microorganisms have been identified to treat high molecular weight humics and kerogens. However, microorganisms have been identified which give substantial degradation of low molecular weight humic and fulvic acids from soils. The application of these organisms to the pretreatment of bauxite ores may be possible.

3 - STAGE 1 - DELETERIOUS EFFECTS OF HUMIC SUBSTANCES ON MINERAL PROCESSING OPERATIONS

3. STAGE 1 - DELETERIOUS EFFECTS OF HUMIC SUBSTANCES ON MINERAL PROCESSING OPERATIONS

3.1 Scope of Work

The project is divided into 3 stages. The scope of work for Stage 1 which is presented is as follows:

(a) Identify and list the mineral processing unit operations adversely affected by humic acids and other naturally occurring organic substances and develop a list of keywords. This initial exercise will be based on the accumulated knowledge of the study participants and a comprehensive survey of contacts in the mining industry.

(b) Perform a computerized literature search for published information regarding the deleterious effects of humic acids and organics on mineral beneficiation. This search will include a review of all recent journals covering the mining and mineral processing industry which may not yet be included in the computer data bases being searched.

(c) Retrieve the literature referenced in the search and perform a critical review of the data in regard to

  (i) the nature of the upset conditions
  (ii) frequency of occurrence
  (iii) the nature of the industry
  (iv) the nature of technical, economic and environmental problems to be resolved.

(d) Prepare and submit a draft interim report for Stage 1.

3.2 Background

3.2.1 Origin of Humic Substances

Humic substances are natural organic compounds which are formed from the partial decomposition of vegetable matter. They occur in natural waters (Hutchinson, 1957; Nordell, 1961; Shapiro, 1964); ground soils (Garrels, 1973; Steelink, 1977; Black, 1963); peat, lignite, coal (Manahan, 1978); sedimentary and carbonaceous deposits (McDonald et al, 1980; Szalay, 1964; Afenya, 1976, Radtke et al, 1970; Zaitseva et al, 1973; Osseo-Asare et al, 1984; Chetverikova et al, 1971). These are probably the most extensively distributed natural organic matter.

3.2.2 Nature of Humic Substances

Humic substances are a major organic fraction in natural waters and soils. They are amorphous, brown or black coloured, hydrophilic, acidic, polydisperse, geopolymeric substances of molar mass ranging from 10^2 to 10^5.

The humic substances are classified into three main fractions based on the solubility in alkali and acid (Black and Christman, 1963; Schnitzer and Khan, 1974; Mallevialle, 1974). They are classified as:

(a) Fulvic Acid (FA) - soluble in both acid and alkali.

(b) Humic Acid (HA) - soluble in alkali and insoluble in acid and ethanol.

(c) Hymatolmelanic Acid - Soluble in both alkali and ethanol but insoluble in acid.

(d) Humin - insoluble in both alkali and acid.

Further classification of humic acid is possible. Figure 3.1 summarizes humic substances' fractionation. The fractions FA, HA, and Hymatolmelanic acid possess similar structure, but they differ in molecular weight, ultimate analysis and functional groups. The fulvic acid has low molecular weight, but the oxygen content in the functional groups per unit mass is higher (Schnitzer and Khan, 1974).

All humic fractions exhibit resistance to microbial degradation and thus these are termed refractory. Helfgott, et al (1977) assessed the biodegradability of humic matter in an aerobic environment cultured with domestic sewage organisms. They observed that the humic fractions resist degradation during 30-day incubation period.

The synthesis of humic substances, known as the humification process, has not yet been clearly understood. Felbeck (1971) proposed four theories of humic substances formation; however, it is not explicit which mechanism prevails. The most logical hypothesis is that the more complex high molecular weight humic matter is formed first, and then these are degraded oxidatively to low molecular weight compounds.

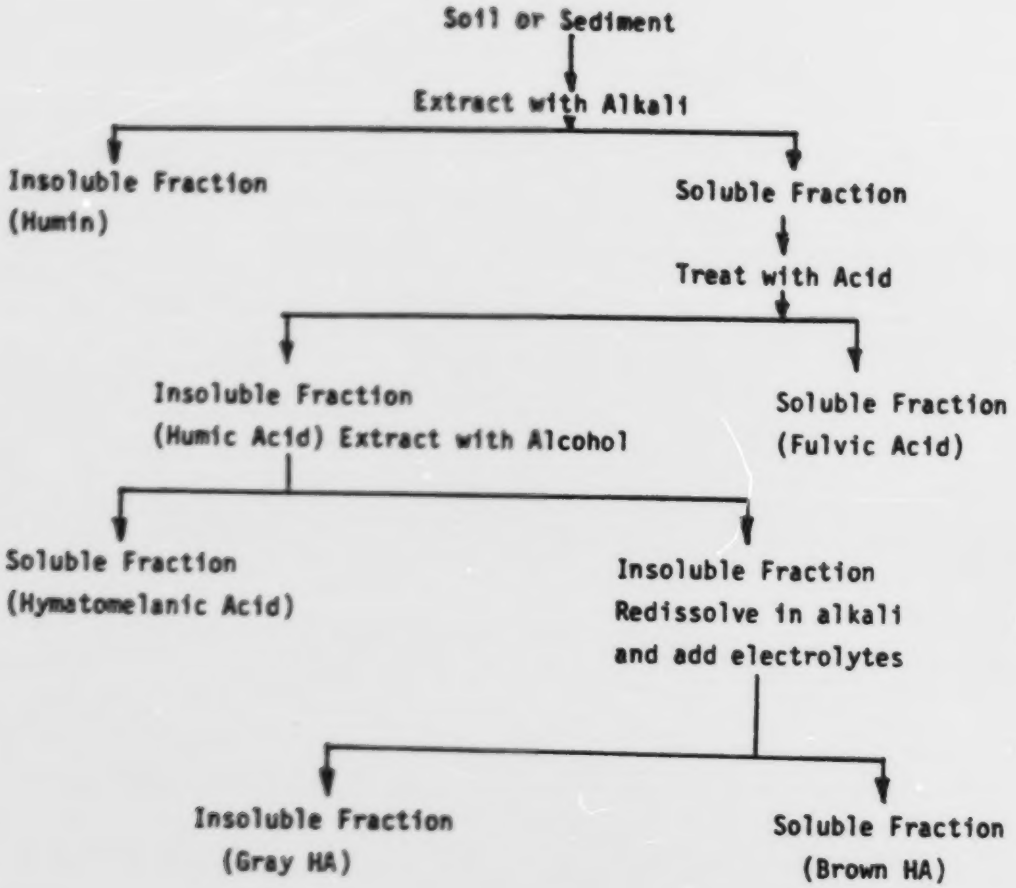


Figure 3.1 Fractionation of Humic Substances
(From: Schnitzer and Khan, 1972)

3.2.3 Chemical Characterization

Humic substances are primarily composed of carbon, oxygen, hydrogen, nitrogen and sulphur. Schnitzer and Khan (1978) summarized the humic and fulvic acids in various soils as shown in Table 3.1.

Table 3.1 - Elemental Analysis of Soil Humus

Element	Humic Acid (%)	Fulvic Acid (%)
Carbon	53.6 - 58.7	40.7 - 50.6
Oxygen	32.7 - 38.3	39.7 - 49.8
Hydrogen	3.2 - 6.2	3.8 - 7.0
Nitrogen	0.8 - 3.5	0.9 - 3.3
Sulfur	0.1 - 1.5	0.1 - 3.6

Gjessing (1976), Black and Christman (1963), and Weber and Wilson (1975) summarized the elemental analysis of the aquatic humic and fulvic acids. This is presented in Table 3.2.

Table 3.2 - Elemental Analysis of Aquatic Humus

Element (%)	Aquatic Humus (%)	Humic Acid (%)		Fulvic Acid (%)	
	Gjessing (1976)	(B&C)	(W&W)	(B&C)	(W&W)
Carbon	43	46.63	53.4-59.5	40.6- 41.5	45.7-51.1
Oxygen	N.A.	46.83	N.A.	49.78-50.80	N.A.
Hydrogen	5.5	5.22	3.73-5.11	5.45- 5.72	3.62-4.26
Nitrogen	1.1	1.32	2.10-1.95	1.53- 1.98	1.13-1.57

(B&C): Black and Christman (1963)

(W&W): Weber and Wilson (1975)

The carbon content in the humic acid is greater than in the fulvic acid while the oxygen content is higher in fulvic acid.

Humic substances on burning yields an ash content which is indicative of inorganic compounds associated with the organic molecules.

The major oxygen containing functional groups in humic substances are carboxyls (-COOH), phenolic and alcoholic hydroxyls (-OH), carbonyls (-C=O) and methoxyls (-OCH3). Most of the oxygen can be accounted for by these functional groups, in humic acid (63-91%) and fulvic acid (>90%), (Schnitzer and Khan, 1972). Table 3.3 presents data on oxygen containing functional groups in humic substances.

Table 3.3 - Oxygen containing Functional Groups in Humic Substances (meq/g).

	Total Acidity	Carboxyls	Phenolic OH	Alcholic OH	Carbonyls	Methoxyls	
Soil HA	6.7	3.6	3.9	2.6	2.9	0.6	Schnitzer and Khan (1978)
Soil FA	10.3	8.2	3.0	6.1	2.7	0.8	
Water HA	8.2	4.5	3.7	N.A.	4.3	N.A.	Weber and Wilson (1975)
Water FA	10.6	6.8	4.3	N.A.	4.3	N.A.	

The acidity of humic substances is mainly due to the dissociable hydrogen present in the carboxylic and phenolic groups.

Humic substances are chemically complex organic material; the structure of this substance has not been identified. A number of degradative methods have been used to identify humic substance, which included oxidations in alkaline and acid media, reduction, hydrolysis, thermal, radio chemical and biological methods. Schnitzer and Khan (1972) identified phenolic and benzene carboxylic acids as major degradation products of humic and fulvic acids. Thus, the humic substances are not single macromolecules but associations of molecules, the "building blocks". It is postulated that the structure of humic substances is flexible, perforated by voids of varying dimensions that can hold or adsorb organic and inorganic compounds. Figure 3.2 shows the proposed chemical structure of fulvic acid.

The structural flexibility allows the "building blocks" to aggregate or to disperse depending on the pH, ionic strength and the pressure of metal ions. Humic substances at low pH tend to aggregate forming stretched bundles of fibres. Due to increased ionization of carboxylic and phenolic OH groups at increasing pH, the cohesive forces become weaker and the molecular arrangement becomes small and better oriented (Schnitzer, 1980).

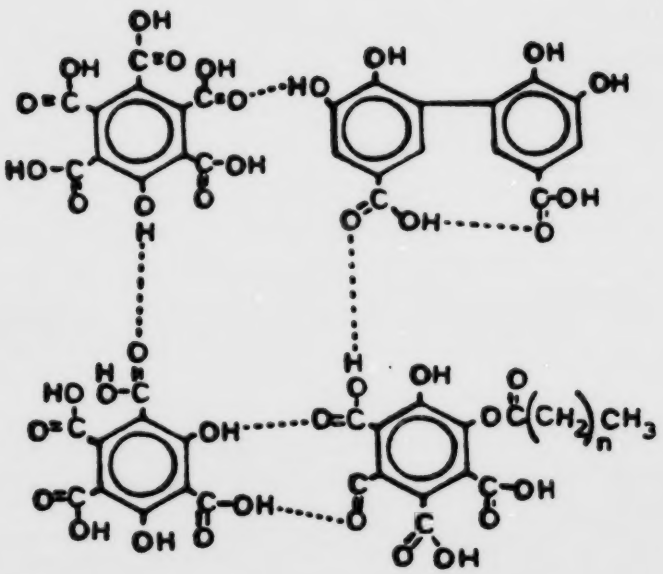


Figure 3.2 - A partial chemical structure of fulvic acid
(From: Schnitzer and Khan, 1978)

3.2.4 Physical Characterization

Humic substances are considered to be hydrophilic colloids because of their large molecular size. Due to the dissociation of carboxylic and phenolic groups, the humus particles are negatively charged under normal water conditions (Black and Christman, 1963).

Many methods have been used to determine the molar mass of humic substances such as osmotic pressure, cryoscopy, diffusion, isothermal distillation, viscosity, gel filtration, sedimentation and ultra centrifugation. These methods produced widely different results eg. chemical methods yield a molar mass of 1300 to 13,000 for humic acids; osmometry, cryoscopy, diffusion and viscosity methods yield values of 700-26,000 for HA and 200 - 399 for FA; ultracentrifugation and light-scattering yield 30,000 to 80,000 (Orlov et al 1971). These discrepancies are due to the poly dispersion of humic substances, aggregate instability, ash content, pH values and ionic strength.

Humic substances fluoresce under visible and ultraviolet light and this property has been used for the quantitative estimation of humic compounds (McCreary and Snoeyink, 1980).

3.2.5 Interaction with Metal Ions

Humic substances form complexes with metal ions. This occurs between the oxygen containing functional groups and the metal ions such as Ca, Mg (alkaline earths), Cu, Fe, Cd, Zn and Ni (transition metals). Monovalent cations such as Na or K form weak electrostatic bonds with the anionic group of the humic molecule. These complexes affect the biological availability and toxicity of the metal ions, and change their geochemical mobility. The stability of humic matter and metal ion complex formation depends on the source of the humic matter, the pH, ionic strength and solution temperature (Saar and Weber 1982).

Schnitzer and Kerndorff (1981) investigated the solubility of fulvic acid and 11 metals in water over a pH range 4 to 9. The solubility of metal ions in water was greatly affected in the presence of fulvic acid (over 200mg/L) by the formation of soluble complexes. The fulvic acid over the pH range 5 to 7, showed greater tendency to form water-insoluble complexes with Fe^{+++}, Al^{+++}, Cr^{+++}, Pb and Cu. Chelation, inner and outer complex formation, adsorption, and co-precipitation were considered as the interaction mechanisms for insoluble complex formation.

Schnitzer (1980) describes these group interactions with the metal ions according to the following scheme:

(a) when COOH group reacts with metal ions, form organic salt.
(b) when COOH group and the adjacent OH group react simultaneously with the metal ion, form a chelate.
(c) when two adjacent COOH group react with the metal ions, form chelate.

3.2.6 Chemical Degradation of Humic Substances

Humic matter isolated from soils has been extensively studied (Barron 1981; Felbeck 1971; Kononva 1976; Ognor 1973; Schnitzer 1978; Steelink 1963). Further investigations on chemical degradation of soil humic acid have shown evidence of both aromatic (Schnitzer and Khan, 1978) and aliphatic (Baron, 1981) constituents. Jakab (1963), et al discovered over 30 phenolic compounds from soil humic acid by hydrolyzing in 5N sodium hydroxide at 170°C. Neyroud and Schnitzer (1975), produced fatty acids, phenolics and benzene carboxylic acids by four successive hydrolysis of humic acid in 2N sodium hydroxide at 170°C. More recently Lever (1978) attempted to characterize the bauxites humic matter to study the problems caused by the individual compounds or fractions in the Bayer process.

He characterized the organics into three main groups:

(a) the humic matter composed of freshly extracted high molecular weight fraction and its degradation products of molecular weight over 500.

(b) the intermediate degradation products, called "building blocks", which make up the large humic molecules, eg. benzene carboxylic acids, phenolic acids, and aliphatic acids.

(c) the low molecular weight aliphatics, eg. formic acids, acetic acids, lactic acids, oxalic acids, and succinic acids.

Recently, Liao et al (1982) made attempts to characterize natural aquatic humic substances (humic and fulvic acids) from two geographically different natural water sources by subjecting them to controlled chemical degradation. Table 3.4 summarizes the identified products into six groups according to the structural similarity.

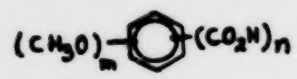
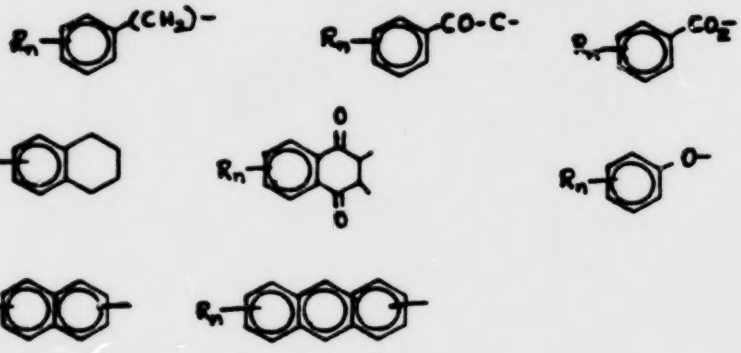
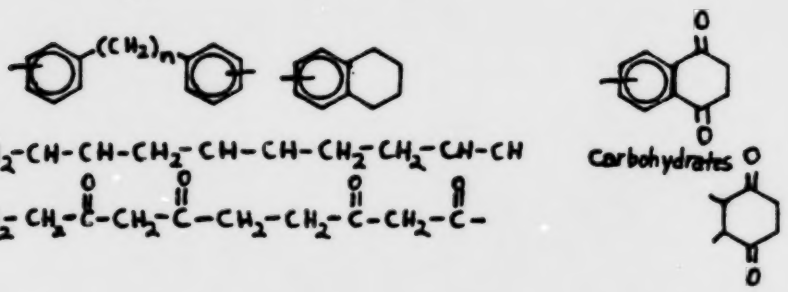
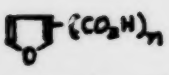
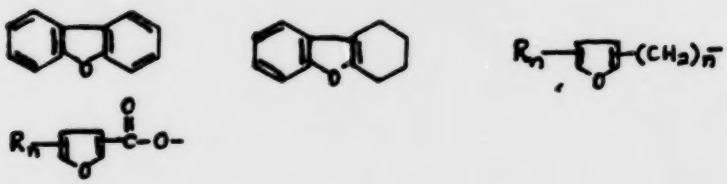
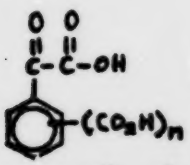
Table 3.5 represents structures of degradation products of aquatic humics and their possible sources in Humic Macromolecules.

Table 3.4 - Summary Results of $KMnO_4$ Oxidation and NaOH Hydrolysis of Aquatic Humic Acids and Fulvic Acids from Black Lake and Lake Drummond. Yields (mg) Resulted from 1.0 g of Starting Humic Samples

	Black Lake				Lake Drummond			
	$KMnO_4$ oxid		NaOH hydr		$KMnO_4$ oxid		NaOH hydr	
components	HA	FA	HA	FA	HA	FA	HA	FA
benzenecarboxylic acid methyl esters	142.71	127.77	5.36	5.56	110.71	104.14	2.96	4.03
furancarboxylic acid methyl esters	13.61	9.98	0.14	0.16	21.10	26.64	0.36	0.38
(carboxyphenyl)glyoxylic acid methyl esters	4.49	4.70	0.00	0.00	5.77	5.61	0.00	0.00
aliphatic monobasic acid methyl esters	4.07	0.82	0.72	2.49	14.84	6.14	0.54	1.99
aliphatic dibasic acid methyl esters	47.57	56.48	10.62	4.57	98.06	98.07	11.30	5.66
aliphatic tribasic acid methyl esters	0.62	1.47	0.29	0.39	4.94	4.52	0.45	0.51
sum	213.07	201.22	17.13	13.17	255.42	245.12	15.61	12.57
identified percentage of total GC peak area	88%	91%	71%	73%	86%	88%	63%	69%

Abbreviations: FA, fulvic acid; HA, humic acid.

Table 3.5 - Summary of Degradation Products and Their Possible Sources in Humic Macromolecules. (Liao et al, 1982)

Type of Degradation Products	Possible Sources in Humic Macromolecule (S)
 benzene carboxylic acids	
oxalic acid malonic acid Succinic acid	
 furan carboxylic acids	
 Carboxyphenyl Glycolic Acids	and isomers
CO_2 Carbon dioxide	Carbohydrates phenols Quinones and others

R = H, OH, CO_2H, or alkyl substituents

Liao et al (1982), based on their degradation studies, postulated that the general molecular structure of aquatic humics may consist of:

(a) single aromatic ring with three to six carboxylic acid, ketone or hydroxyl groups attached to the alkyl side chain.

(b) short aliphatic carbon chains.

(c) polycyclic ring structures which include polynuclear aromatics, polycyclic aromatic - aliphatics, and fused rings such as furan and pyridine.

Although no definite structural relationship has been established between these fragments, it is believed that these fragments are connected with the humic macromolecules through carbon - carbon linkage.

3.3 Conventional Methods of Humic Matter Removal

Humic substances in water are generally removed by coagulation and adsorption processes. Inorganic coagulants and organic flocculants have been employed to remove dissolved organic substances in water treatment plants (Robinson, 1979), while powdered carbon, granular carbon, and resins have been used as adsorbents for the removal of dissolved organic substances in water treatment plants. These conventional methods are discussed below:

3.3.1 Inorganic Coagulation of Humic Substances

Aluminum and ferric salts are most extensively used as chemical coagulants for humic substances removal. On an equivalent basis, these two coagulants exhibit similar behaviour. Hall and Packham (1965), Kavanaugh (1978) and Semmens et al (1979) have studied in detail the characteristics of coagulation in conjunction with sedimentation and filtration.

These studies concluded that the types of organic matter and coagulant, coagulant dosages, and the solution pH are important parameters in the removal of organic matter by chemical coagulation. The extent of removal depends on the type of organic matter present in the water. Kavanaugh (1978) found that humic acid and fulvic acid removal from the water were between 60 to 90% and 10 to 60% respectively. Due to higher density of the acid functional group in fulvic acid than in humic acid, larger dosages of aluminum will be required to increase its removal (Hall and Packham, 1965).

Both the ionization of the functional groups in humic acid and the hydrolysis of the aluminum ions are affected by the water pH. An optimum pH for aluminum coagulation of humic matter ranges between 5 and 6. Hall and Packham (1965) found that the optimum pH for ferric coagulation lies between 3.5 and 4.5 which is due to the more acidic nature of the ferric ion and its affinity for hydroxyl ions.

Maulding et al (1968) investigated the effect of cations such as Na^+, Ca^{++}, and Mg^{++} on ferric coagulation of coloured natural waters at optimum pH. The presence of these cations increased the effectiveness of colour removal in order of Mg > Ca > Na. Maulding et al (1968) also studied the effect of Cl^- and SO_4^{--} anions; SO_4^{--} ions showed a deleterious effect on colour removal at acidic pH.

3.3.2 Polymer Coagulation of Humic Substances

The major role of organic polymer in liquid/solid separation is to enhance flocculation and increase settling rates. Humic matter, being negatively charged under ordinary conditions, can be removed by cationic polymer. The mechanism of humic matter removal from water by cationic flocculants is essentially the result of charge neutralization (Narckis and Rephun, 1977; Glaser 1978).

The effect of pH on the efficiency of cationic polymer is similar to inorganic coagulants. The pH affects the ionization of the functional groups of both the humic molecule and the polymer (Diamodopoulos, 1984). Higher pH enhances the ionization of the humic functional groups, which consequently requires larger polymer dose for effective coagulation. At low pH, the flocculant demand is reduced (Narkis and Rebhun, 1977). The effect of cationic polymer on humic acid and fulvic acid in solution is similar; however, the humic acid coagulates more efficiently than fulvic acid (Amy and Chadik, 1983).

Edzwald et al (1977) investigated the effect of alum and polymer together on the removal of humic substances from water. This treatment scheme proved advantageous over using alum or polymer alone and resulted in the reduction of coagulant doses, operation at higher pH, and the production of large flocs that settle readily.

Weber et al (1978) and McCreary and Snoeyink (1979) studied the effect of pH and observed that the solubility of humic substances decreases with the decrease in pH. However, the humic substances were more readily adsorbed on activated carbon.

Zogorski's (1975) investigations indicated that the carbon characteristics such as particle size, surface area, chemisorbed oxygen, hardness and uniformity, and pore structure are important in the adsorption of organic molecules from solution. These characteristics depend on the source of the carbon and the activation process used. Weber et al (1977) and Snoeyink et al (1977) investigated different types of carbon to study the commercial humic acid adsorption from water. The carbon type "F-400" was chosen for study because it had better adsorption rates and capacities and had superior resistance to abrasion. They found that the rate of adsorption for humic acid was inversely proportional to the particle size.

Wilson (1960) investigated the removal of fulvic acids from water on a full-scale plant using granulated activated carbon (GAC) bed. Fulvic acids were oxidized by adding hydrogen peroxide prior to the treatment. For a 50% break through of about 2 ml/l of fulvic acid, the service time for the carbon bed was 3 months at a flow rate of 3 gpm/ft^2, and the removal efficiency decreased from 100% to about 20%. Wilson inferred that the decrease in the GAC adsorption efficiency was due to the larger size of the organic molecules relative to the average pore size of the carbon.

It has been observed that the fresh GAC removes organic matter effectively just after starting but becomes less effective with time. Therefore, coagulation of as much organic matter as possible prior to GAC adsorption in a water treatment plant should result in a lower overall treatment cost.

Sontheimer and Maier (1972) clarified Rhine river water with aluminum sulphate and ferric chloride prior to passing through GAC beds. They found that the adsorbability of organic substances by GAC beds significantly increased by prior coagulation.

The literature review on the removal of humic substances from water indicate that the humic substances from different sources behave differently. The extent of humic substances removal by coagulation greatly depends on the type of the organic matter. The performance of coagulation before adsorption may result in an increase in carbon adsorption efficiency, but the adsorption also varies with the source of humic substances and the carbon characteristics.

3.3.3 Resin Adsorption of Humic Substances

The removal of humic substances from water by coagulation and adsorption on activated carbon has been discussed in the foregoing sections. This section is intended to show effective removal of organic matter by resinous adsorbents. Numerous studies (Abrams et al 1965, Tilslay 1978, Cameron 1983) have been made to alleviate the organic fouling problem. Abrams (1964) reported two basic requirement for a good scavenging resin (a) high adsorption capacity for humic substances, (b) good regeneration capability. Bohnsack (1963) investigated 37 different European and American resins to determine their adsorption characteristics for humic acids. Depending on the resin composition and the initial concentration of humic acids in water, 3% to 98% adsorption efficiencies were obtained.

Also, Type I, strong-base resins, had greater adsorption capacity for the organic substances than Type II, weak base resins, but the latter were more readily regenerable with caustic soda.

Ward and Edgerley (1965) made a significant contribution by showing that a Type I strong-base resin was fouled not only by high molecular weight compounds but by simple organic acids such as benzoic acid and its derivatives. Abrams (1964) reported that a resin must possess three basic features for humic acid (anionic detergent) adsorption from water: (a) weak-base groups, (b) hydrophilic matrix, and (c) macroporous structure.

Most strong-base resins have high affinity for humic acids but do not release these acids on either alkaline or salt regeneration. Weak base resins also adsorb these acids to various degrees but they can be reused repeatedly because they can be regenerated with alkali. The macroporous phenolic weak-base resins are found most suitable. The Duolite A-6 and A-7 resins can be regenerated 100% if the organic acid loading is limited to 50% to 60%.

3.3.4 Alternate Process of Humic Substances Removal

3.3.4.1 Ozonation

Ozone is a toxic gas; it is generated on demand at low pressures and concentration for water treatment application. The ozone is a strong oxidant and is used to reduce the Total Organic Carbon (TOC) concentration. It increases biodegradability of the organics present in the water at relatively low cost. Because of its high cost and low dosage requirement, it is mainly used for drinking rather than waste treatment. The basic mechanism by which the ozone reacts with the organic materials are (Stephenson 1979):

(a) by addition to the aliphatic unsaturated compounds.
(b) by substitution of one atom or functional group.
(c) by oxidation of the organic molecule with or without degradation of the organic compound.

Hoigne and Bader (1976) have shown that ozone may react directly with the organic substrate or decompose prior to reaction to produce hydroxyl radical °OH which may be more reactive than ozone itself.

Humic substances are relatively more resistant to ozonation than many other organic compounds. A summary of ozone identified reaction products is presented in Table. 3.6. Extended ozonization results in the formation of acetic acid, oxalic acid, formic acid, and terephthalic acids, phenolic compounds and carbon dioxide.

In water treatment plants, ozone is applied prior to granular activated carbon adsorption columns (Rice, et al 1979) to improve the organic material removal (Sontheimer, et al, 1978, Khun et al, 1976). Rice et al (1979) observed that the organic removal is greater when ozonation is followed by carbon adsorption than by the individual effect of ozonation or carbon adsorption. It is believed that the combined effect of ozonation and carbon adsorption has increased biological activity. Eberhardt, et al 1975) investigations have shown that the activated carbon removed greater TOC (3 mg/l) after ozonation than before ozonation (1 mg/l TOC).

TABLE 3.6

REACTION PRODUCTS AFTER OZONATION OF AQUEOUS SOLUTIONS CONTAINING HUMIC SUBSTANCES (HS)

Reference	Humic S Conc. (mg/l)	Expt. Media and Reaction Volume	Gas Flow and Ozone Conc.	Contact Time or Applied O_3 Dose	Reaction Products Identified
Ahmed and Kinney, 1950	1000-2000	4% KOH 150 ml	15 l/hr 56 mg/l	0-48 hr.	Benzene carboxylic acids, acetic and oxalic acids, CO_2 carbonic and oxalic acids the major products - accounts for 65% of the remaining TOC, the rest being colourless, water soluble, ozone resistant acids.
Kinney and Friedman, 1952		alkaline aqueous			Oxalic acid, terephthalic acid, small amounts of acetic acid, and CO_2
Sherzhenko and Taran, 1966	1000	aqueous		1.3 hr.	Oxalic, acetic, and formic acid, carbon dioxide
Daydens, 1970	river water	neutral aqueous		0.87-1.58 (mg/l)	Higher suspended solids, phenol, and C.O.D. - possibly from fragmentation by decomposition of Fe and Mn from organic ligands
Malleviallie, 1975 et al., 1976	100-200	neutral aqueous 2.5 l	66 l/hr 24 mg/l	0 - 2 hr.	Phenolic fragments, formic acid; Release of pesticide Lindane detected. Metal complexes liberated (Mn). Noted similar products formed by ozonation of salycilic acid. 0.19 mg O_3/mg HS required for 95% colour reduction, 0.61 mg O_3/mg HS required for 95% polyhydroxyaromatic derivatives elimination 0.72 mg O_3/mg HS for 75% COD reduction 0.95 mg O_3/mg HS for 75% TOC reduction
Lawrence, 1979	1000, and 'high humic' raw water	neutral aqueous 500 ml	120 l/hr.	1 hr. 475 mg/l	Colour removal, lower MW compounds including branched, substituted, and simple dicarboxylic acids, low MW straight chain and branched acids, oxalic and acetic acids, phenoxy ketones and aromatic and nitrogen compounds.
Rao, et al., 1976	oxalic acid 40-50 mg/l	neutral aqueous	280 l/hr. 25 mg/l	0 - 2 hr.	Acetic acid oxidized to glyoxylic acid and then to oxalic acid slow oxidation of oxalic acids to CO_2

In the ozonation of concentrated solutions of humic substances, greater than 200 mg/l HS, found in surface waters, Mallevialle et al (1978), and Hartemenn, et al (1978) observed that the biochemical oxygen demand (BOD_5) increased after ozonation.

The ozone pretreatment effects on the biodegradability of refractory humic substances found in sources of drinking water may be more directly related to the ozonation practices typical of most water treatment plants.

3.4 Literature Search and Industry Contacts

3.4.1 Literature Search

A computer literature search, utilizing the DIALOG, CAN-OLE and ORBIT bases, containing Chemical, Metallurgy and Engineering Abstracts, was carried out. Groups of key words were formed from: Humic Acid, Uranium, Solvent Extraction, Hydrometallurgy, Sedimentary Deposits.

A very limited number of papers pertaining to the mining industry were provided. It is indicative of the level of research on the effects of humic acid on processes in the mining industry.

3.4.2 Industry Contacts

As a result of the limited information available in the literature, several mining companies, equipment manufacturers, reagent suppliers, institutions, agencies and selected consulting engineering firms were contacted. A list of these contacts is given below:

Mining Companies

Exxon
Alcan
Noranda
Inco
Falconbridge
Sherritt
Kidd Creek
Dome
Kennecott
Gulf
Sohio
Homestake
Freeport
Eldorado
Rio Algom

Vendors - (Equipment Suppliers/Reagent Manufacturers)

Envirotech
Dorr Oliver
Himsley
Calgon
Rohm and Haas

Vendors - (Equipment Suppliers/Reagent Manufacturers) - (cont'd.)

Duolite
Henkel Corp
Dow
Cyanamid
Interox

Institutions and Agencies

CCIW
USBM
NIM. (S.A.)
SRI
McMaster University

Consultant Engineers

Kilborn
SNC
Fluor
A.H. Ross

The information gained from these contacts was mainly verbal and is continuing and is presented under the section of discussion of problems.

3.5 Discussion of Problems

Mining companies were asked about their experiences with deleterious effects on mineral processing plants of humic substances or organics present in their ore-bodies. As a result of these conversations, some of the important problems faced by the mining industry in unit processes such as liquid/solid separation, ion-exchange, solvent extraction and flotation are discussed in Section 3.5.3. Those contacted indicated that upset process conditions were generally considered to be caused by humic substances or their degradation products and often the effects were seasonal.

Except for some isolated cases in the uranium and aluminum industries, which are discussed in Section 3.5.3, research and development studies in the areas of humic acid influence on minerals processing circuits have not been undertaken. Discussion relating to the mining industry are therefore based on personal communication.

3.5.1 Water Treatment Plant

Humic substances impart yellowish-brown colour to the natural water, rendering it undesirable for human consumption due to aesthetic reasons (AWWA Joint Committee Report, 1970). In water treatment plants, chlorination of water produces haloforms at significant levels (Rook 1974) which could cause cancer at the proposed level of 100 g/L for total trihalomethanes (AWWA Committee Report, 1979).

3.5.2 Aquatic Environment

Humic substances react strongly with metal ions and organics, and change the solubility of them significantly. They affect their mobilization in aquatic environments (Schnitzer and Khan, 1972). These reaction products of humic substances with the heavy metals and/or toxic organics (eg. pesticides) affect both the water and the environment surrounding the water.

3.5.3 Minerals Processing

In the mining industry, humic acid or organic problems in processing are caused by either the mine water or by humic acid, its degradation products and organics in the ore.

At Kidd Creek Mines in Ontario, humic acid related problems are encountered in pyrrhotite flotation. It is believed to be due to the recycle water. At Sohio, the presence of organic carbon in the ore is a factor in processing problems.

In aluminum production at Alcan and Kaiser, the organic carbon content of bauxite is considered to be responsible for liquid/solid separation problems.

3.5.3.1 Liquid/Solid Separation in Aluminum Production

Organic carbon, from 0.1 to 0.6% by weight, as humic, cellulose and protein has been found in bauxite ore (Gnyra et al 1979, Guthrie et al 1984, Lever 1978).

At Alcan, during digestion with the Bayer process, over 50% of the organic carbon (low molecular weight fraction) is extracted into the liquor, as are 25% in both the high and intermediate fraction. Sodium oxalate concentration in Bayer liquor gradually rises to an equilibrium-saturation level. Further saturation causes sodium oxalate crystallization, creating problems such as poor classification - settling of fine Bayer seeds and excessive scaling in process lines and tanks. Alcan is considering the development of an economically viable process to treat the ore to remove humic acid to avoid formation of degradation products.

Personnel at Dorr-Oliver, a major supplier of process equipment, have also expressed concern about the sodium oxalate crystallization, which causes filtration problem.

Kaiser Aluminum is encountering problems similar to Alcan. They have initiated discussions with the US Bureau of Mines about studies in the field of humic acid removal.

3.5.3.2. Ion-Exchange Circuit

The presence of humic substances in sedimentary sand, silt stone (rolifront deposits) and carbonaceous deposits are the cause for detrimental effects in various unit operations for the extraction of metals. (McDonald et al 1980).

In uranium extraction, the organic compounds in the ore are readily dissolved during leaching, becoming part of the pregnant solution. In the ion-exchange stage of the process, the dissolved organic contaminants poison the resin, decreasing the available reaction sites for uranium adsorption.

The field of resin poisoning is of a great concern to the uranium industry. Consultants such as A.H. Ross and suppliers such as Himsley recognize the need for work in this area.

3.5.3.3 Solvent Extraction Circuit

Humic acid and other organic substances in uranium ore deposits, when extracted in the organic diluent, are one of the causes of emulsion and crud problems in the solvent extraction stage. One reason is believed to be a reaction between amines and humic acid. The Henkel Corp., a supplier of amines for uranium extraction, with consultants A.H. Ross have investigated this problem at Sohio. Low value pregnant strip solution could also be caused by an amine -humic acid reaction.

Degradation products of humic substances form insoluble salts causing crud formation. Studies of this effect have been conducted by McDonald et al, and detailed studies conducted by Ritcey (CANMET). Further discussion of humic acid related solvent extraction problems will be made in a later report, when additional material has been received.

3.5.3.4 Flotation Circuit

Problems believed to have been caused by humic acid have been encountered in the pyrrhotite flotation circuit at Kidd Creek Mines. Mill staff describe the problem as seasonal, i.e. occurring during spring run-off season. It is assumed to be a process water problem.

In column-flotation studies, humic acid content in process water has been identified as the cause for upset process problems.

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4 - STAGE 2 - BIOLOGICAL METHODS FOR THE DESTRUCTION OF HUMIC SUBSTANCES

4.1 - Introduction

In the first report of this study, it was confirmed that organic compounds (and more particularly humic substances and oxalates) are perceived to interfere in specific mineral processing operations. The problems include:

- Difficulties with pyrrhotite flotation (Kidd Creek Mines)
- Poisoning of ion-exchange resins in uranium processing
- Emulsions and crud formation in solvent extraction (Sohio)
- Problems in aluminum processing by the Bayer process due to oxalate formed as a product of the oxidation of humic substances in bauxite ores. The problems include difficulties with liquid/solid separations and crystallization of sodium oxalate in the processing circuits. (Aluminum Company of Canada (Alcan), Kaiser Aluminum and other aluminum processors).

In one case (Kidd Creek), the problems tend to be seasonal, i.e. due to variations in the concentrations of humic substances in ground waters etc. at different times of the year. However, the problems encountered by the aluminum processing industries relate to the presence of geopolymers (humic substances) as well as biopolymers (proteins, polysaccharides, lignins, fats, and waxes) in the bauxite ores.

Alcan researchers have identified that during the Bayer process by which the bauxite ore is processed, trace amounts of humic substances are converted to either mono- or di- aliphatic carboxylic acids. Thus, organic acids such as acetic, formic, oxalic, succinic and malonic are found in the process liquor in addition to low molecular-weight fractions from the humic materials (Lever, 1978; Guthrie et al., 1984). The objectives of this section of the study are to investigate the ability of micro-organisms to degrade these humic and organic materials and to assess the technical feasibility of using them to prevent problems attributed to these organics in mineral processing. To this end, a literature search has been conducted.

4.2 - Scope of Work

The scope of work for Stage 2 is as follows

(a) Define the humic compounds and/or mineral by-products operations for which biological processing destruction methods are being sought. The selection of key compounds for the initial literature search is guided by the conclusions derived from Stage 1. Humic substances refer to a complex, at times poorly defined, group of compounds. A broad list of keywords is developed reflecting this lack of consistent definition.

(b) Perform a computerized literature search for information relating to the microbiological destruction of target compounds. The successful computerized search methods depend on the keywords conforming with topic words in titles or abstracts. This is not always the case and a manual literature check is also required. This follow-up check includes an expanded search for information on key microbial genera or species identified in the computer search.

(c) Contact research groups actively studying the degredation of humic substances. Data published in the literature is somewhat outdated due to the time that is required to get it to print. Any research groups identified as actively working in this or related areas will be contacted (telephone or letter) to up-date the published information (provided the researchers are willing to co-operate with us).

(d) Retrieve and critically evaluate available information on each organism or mixed population including

- the identity of the organisms (pure or mixed cultures)
- specific compounds degraded or feedstocks used
- nutrient requirements
- conversion efficiencies

- by-products
- reaction/growth rates
- metabolic mechanisms
- toxicity problems
- level of development (bench, pilot scale etc.)

(e) Prepare and submit a draft interim report for Stage 2.

4.3 - SUBSTRATES

Of major relevance to this study is the level of information available on the composition of the interfering organics as they occur in mineral processes. In the case of oxalic acid and other organic acids (or their salts) in the Bayer process liquor, this characterization has been well documented (Lever, 1978; Guthrie et al., 1984). Organic matter including geopolymers such as humic substances (Reuter & Perdue, 1977) and biopolymers such as lignin, cellulose and protein are said to be present in the bauxite ore. Formic, oxalic, acetic, succinic, propane tricarboxylic and benzene tetracarboxylic acids as well as humic substances were identified in the plant liquors (Lever, 1978). All of these acids have long been known to be formed as by-products of the alkaline oxidation of humic substances. The amounts of these materials in the two process liquors investigated by Guthrie et al. (1984) varied quite substantially. Although the carboxylic acids are well characterized, the data on the humic fraction are much less detailed. The variation between the liquors from the two plants selected by Guthrie et al. (1984) as to their relative concentrations of each carboxylic acid probably arises from the differing compositions of the humic substances in the ores as well as possible variations in the processing conditions.

Caution is necessary therefore, in attempting to correlate the data from published studies to a potential application in a specific mineral processing situation. Most of the investigations to the present time have studied soil or occasionally aquatic humic substances. The precise nature of the humic substances in ore deposits are not known at the present time. The source of a humic substance, i.e. whether it is derived from terrestial or marine organic material, will influence its composition and hence its susceptibility to biological degradation. The complexity of making deductions was described very succinctly by MacCarthy and Malcolm (1979) in a presentation advocating the establishment of a reference collection of humic substances viz;

> * The problem becomes vastly more complicated when the substrate varies from worker to worker as in the case of humic acids, and where there has been no standardization of extractive, fractionation or purification procedures. It is thus virtually impossible to objectively and critically compare the laboratory results of different workers in this interdisciplinary area of research.*

Thus, the precise chemical identity of a humic substance as used by a researcher will be influenced by its origin (i.e. whether the biological material from which it was formed was of predominantly aquatic or terrestial origin) and the method of isolation (i.e. the extent to which the material may have been degraded during the chemical extraction procedure). The climatic conditions under which it was formed and the soil inorganic constituents also influence the chemical nature of humus (Flaig et al., 1975). As MacCarthy and Malcolm note, there is little guarantee that data obtained by one researcher are relevant to those generated at another laboratory using different humus samples and different extraction protocols. Such reservations also apply to the studies on biological degradation. The biological studies commonly use "defined" substrates in that the procedures by which the substrates were isolated and purified are stated. However, thereafter the term is of doubtful value in characterizing the sample and the fact that an organism actively degrades a humic fraction in one laboratory will not guarantee its effectiveness against the same substrate isolated by a different technique.

Humification in soils involves the biological degradation of plant and other organic materials, initially by the action of smaller animals but eventually by the action of bacteria and fungi. Thus proteins, polysaccharides, lignins and waxes etc. are decomposed. During this process the carbon to nitrogen (C:N) ratio of the undigested residue changes markedly. An average value for the C:N ratio in plants is approximately 100 but this is changed as the carbon fraction is preferentially degraded. Thus

when the C:N ratio is approximately 10, the material is considered to represent a mature "humus". The compound at this stage is dark colored and although plant constituents were the original substrates, the precise origin of the components in the final humus is still a matter of considerable debate (Flaig et al., 1975).

In seeking biological procedures to degrade humic substances, it is important to define accurately the nature of the problem. In the soil, the humus acts as an important source of nitrogen to plants. Through its controlled degradation, the organic nitrogen bound-up in the molecular structure or entrapped within the complex is gradually made available to the vegetation. The resistance to degradation (of the humic substances) is obviously a valuable factor in the control of the natural process. Jenkinson (1971) estimated that the annual rate of decomposition of humic substances was 2-5%. That they degrade is well recognized but as Flaig et al. (1975) commented about humic substances "they serve as carbon sources only for special species of organisms". The goal of this study is to identify this special group of organisms and examine what is known about how they degrade humic substances. One perceived strategy will be to find a method to overcome the natural barrier against microbial degradation which is inherent in the molecular structure of the humic substances.

Estimates for the time required for humic components to degrade in the natural environment have been calculated by radiodating (Fukushima, 1982). They are:

Freshly Formed Humus	; 2 - 6 years
Fulvic Acids	; 40 - 120 years
Humic Acids	; 80 - 2000 years

If one assumes that in the case of ore deposits the predominant humic substances will be of the more "mature" age group, it is apparent that the target compounds will be very resistant to biological decay.

4.3.1 - Humic Substances

Scientists have studied humus and humic substances for many years. The fractionation of humus has been based on the differential solubilities of the major components. Such fractionation procedures have long been in existence and although newer techniques are being applied to the problem, the traditional fractionation of humus into fulvic acids, humic acids, humus and hymatomelanic acid is still the best method of separating a complex material into defined fractions (see Table 4.1 "Defined" in this context infers that the separation technique is delineated and that the fractions as classified represent groups of compounds of similar solubility characteristics. In reality, each fraction includes a group of compounds of similar chemical properties. As newer techniques are being developed, e.g. ultrafiltration and column chromatography, the purities of the various fractions are being constantly improved and this will result in more precise and better characterized fractions.

TABLE 4.1

FRACTIONATION OF HUMIC SUBSTANCES

Fractions	Alkali	Acid	Alcohol
Fulvic Acids	Soluble	Soluble	-
Humic Acids	Soluble	Insoluble	Insoluble
Hymatomelanic Acids	Soluble	Insoluble	Soluble
Humins	Insoluble	Insoluble	Insoluble

From Flaig et al. (1975)

The four fractions appear to be structurally related (Schnitzer and Khan, 1972). However, they differ in their elemental compositions, molecular weights and contents of functional groups. The hymatomelanic acid fraction is not considered a major fraction and, in the literature searched to-date, no articles have been identified which relate to its biological decomposition. Based on the polarity and solubility of the fraction in acid and base, it may be surmised that hymatomelanic acid will resemble humic acid in its structure and hence sensitivity to degradation. Humin appears to be a higher molecular-weight relative of humic acid. Since most of the data have been published on humic and fulvic acids, deliberations on the chemical nature of the major fractions will be dominated by the humic acid and fulvic acid components. The term "humus" will be used interchangeably with humic substances to denote unfractionated material.

The elemental compositions of humic and fulvic acids are shown in Table 4.2 (Schnitzer, 1976). Table 4.3 shows the relative ranges of oxygen-containing functional groups in humic and fulvic acids (Stevenson, 1982). Much has been published on precise values for the functional group contents of specific samples. However, in view of the potential error in some of the analytical techniques (Perdue, 1984), the comparison of ranges would appear to be a more practical approach.

Humic acids tend to have a higher nitrogen content than the fulvic acids whereas the latter group of compounds have a higher proportion of oxygen. This is in accord with the data on the oxygen-containing functional groups, which show that the fulvic acids have a higher content of alcoholic and carboxyl groups per gram of solid. As newer purification techniques and better analytical methods become available, the data tend to change but the conclusions which can be drawn from the observed patterns appear to stay constant. Newer non-destructive analytical techniques may be more likely to change theories than will better purification techniques (although the latter advances may simplify the analysts' task).

TABLE 4.2

Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA)[1]

Element	% dry, ash free weight	
	HA	FA
C	50-60	40-50
H	4-6	4-6
N	2-6	< 1-3
S	0-2	0-2
O	30-35	44-50

(1) From Schnitzer (1976)

TABLE 4.3

Oxygen-containing functional groups in Humic and Fulvic Acids[1]

Functional Group	Concentration Range (mmol/g)	
	Humic Acids	Fulvic Acids
Carboxyl	1.5 - 5.7	5.2 - 11.2
Phenolic	2.1 - 5.7	0.3 - 5.7
Carbonyl	0.1 - 5.6	1.2 - 4.2
Alcoholic	0.2 - 4.9	2.6 - 9.5
Methoxyl	0.3 - 0.8	0.3 - 1.2

(1) From Stevenson (1982)

Both humic and fulvic acids behave as polyelectrolytes due to the presence in their molecules of carboxyl and phenolic groups which dissociate at the pH ranges normally found in soils and water. Both fractions have been identified to contain trace amounts of fatty acids, i.e., as much as 10% by weight and also both aromatic and aliphatic components (Ogner and Schnitzer, 1970; Schnitzer, 1976). Based on the maximum yields of the major products from the chemical degradation techniques (approximately 40% by weight of the starting materials were recovered), Schnitzer (1976) calculated that the aromatic components constituted approximately 70% of both fulvic and humic acids. The major difference was that the fulvic acid had a higher content of phenolic hydroxyls.

The methods of studying the chemical composition of these fractions have for many years been based on the controlled chemical degradation procedures followed by identification of the products. These procedures were outlined in the earlier report. From the knowledge of the types of compounds which will yield such products under the same reaction conditions, the researchers were able to develop hypotheses as to the structures of the humic substances from which the degradation products were derived.

The advent of nuclear magnetic resonance (NMR) procedures for the study of humic substances has generated some very important data. Hatcher et al. (1981) examined humic and fulvic acids from an Armadale soil (Prince Edward Island) by cross polarization, magic-angle ^{13}C NMR and confirmed the aromatic nature of these fractions. However, the NMR spectra in many instances led them to conclude that aliphatic components were present in significant amounts, so much so that they speculated that the chemical oxidation/degradation techniques may have caused excessive degradation of the aliphatic constituents. Thus, the aromatic character of the humic substances will have been overestimated. They also noted that the content of carboxyl groups, based on the NMR spectra, was greater than the value calculated from the standard chemical analyses. In fact, the carboxyl content of a humic acid was almost equivalent to the total acidity. If correct, this would mean that

phenolic groups are not as significant contributors to the total acidity as was thought. (In traditional analysis, the phenolic hydroxyl content is calculated as the difference between the total acid groups and the total carboxyl groups as measured. Hence, any error in either of these calculations will give a major discrepancy in the assumed value for the phenolic hydroxyl content).

These observations matched those of Grant (1977) who had proposed that polymethylene chains were major constituents of soil humic materials. His data were also generated by NMR and he believed that the presence of the polymethylene chains would explain the high resistance of the humic materials to chemical and biological degradation. Barron et al. (1980) using NMR with soil samples found that the organic matter could range " from highly aliphatic to moderately aromatic." Earlier, Anderson and Russell (1976) using infrared (IR) spectroscopy and gas chromatographic (GC) techniques had also proposed that soil fulvic acid contained polymeric aliphatic components resembling polymaleic acid.

These data from the newer NMR techniques also matched observations made in 1963 by Barton and Schnitzer. While developing their technique of methylation of fulvic acid followed by mass spectrographic identification of the products, they submitted some methylated extracts to NMR analysis (by less sensitive techniques than currently available). They were surprised to identify no arcmatic or olefinic groups in the NMR spectra of the different methylated fractions. Instead, the major groupings identified were due to aliphatic C-H bonds i.e. methyl ($-CH_3$) or methylene ($-CH_2$) groups.

The above observations suggest that the humic substances may be more aliphatic and hence less aromatic than has been previously thought and that the phenolic contents of the humic substances may be much lower than expected. Instead, carboxylic acids are responsible for most of the dissociable acidic groupings. These hypotheses have still to be confirmed by further experimental data. However, they do provide important information for consideration when attempting to elucidate how selected micro-organisms apparently catalyze the decomposition of the humic molecules. They may also

suggest why the humic substances are refractory against biological degradation. Similarly, they would also explain how under certain oxidizing conditions aliphatic carboxylic acids (e.g. oxalic) can be generated in significant amounts from humic materials.

4.3.2 - Microbial Components of Humus

Another approach to considering the nature of the resistance to microbiological decomposition, which is inherent in humic substances, is to examine current theories of how humus is formed. This is relevant since these compounds refractory towards microbial attack are formed as a result of the biological decomposition of vegetation.

Stevenson (1982) has reviewed the four theories of how humus is formed. The earliest theory propounded by Waksman (1932) is that the humus is derived from lignins. This is based on the high resistance to biological attack shown by natural lignins. Thus, humus is a derivative of lignin which during natural decay processes loses methoxyl (OCH_3) groups, and gains by oxidation processes, o-hydroxyphenol and carboxyl reactive groups. These modified lignins in some way condense with proteins to form the humic substances (the condensation reaction will explain the nitrogen component consistently present in all humic substances). However, Stevenson contends that the lignolytic abilities of soil organisms are not fully appreciated and that for this reason, lignin may not contribute such a significant fraction of the humic substances as once was thought. He does believe that in certain types of environment, e.g. lake sediments, poorly drained soils etc., where anoxic conditions will preclude the active oxidative decomposition of lignin by aerobic micro-organisms, the lignin residues may represent a larger component of the eventual humic material.

A second theory, the polyphenol theory, is, according to Stevenson (1982), the more widely accepted theory at the present time. In this hypothesis, the lignin molecule undergoes demethoxylation and biological oxidation as described in the previous theory (many bacteria can catalyze

these reactions without degrading the lignin molecule which contains significant numbers of methoxyl and aliphatic side chains). However, the polymeric residue (polyphenol) is then converted to quinones by polyphenol oxidase enzymes produced by soil micro-organisms. The quinones are very reactive and condense readily with amino nitrogen compounds to form the humic polymers. This theory is a modification of the lignin theory except that the polyphenols need not originate from the degradation of lignin. Martin and Haider (1971) found that Fungi Imperfecti such as Hendersonula toruloidea, Epicoccum nigrum, Stachybotrys atra, Stachybotrys chartarun, and Aspergillus sydowi will attack both lignin and cellulose with the formation of dark-brown polymeric products, similar in composition to humic substances.

A related microbial synthesis theory infers that the humic-like substances are formed by fungi and actinomycetes in the soil from carbon compounds other than lignin. By their metabolism, these organisms synthesize polyphenols which are subsequently converted enzymatically to humic substances via the quinone route described above. Thus the micro-organisms convert low molecular-weight components to dark-brown polymeric structures (melanins or melandoins). In recent years, several investigators have shown that these by-products isolated from microbial cultures (where no lignin is present), resemble closely humic acids isolated from natural sources (Kang and Felbeck, 1965). The more common organisms for such studies are fungi and actinomycetes, many of which are known to be able to convert aliphatic carbon compounds to aromatic compounds such as phenols and hydroxy aromatic carboxylic acids.

The final theory contends that the brown polymers are formed by a non-enzymatic browning reaction between reducing sugars and amino acids. Such a reaction occurs in foods as they become dehydrated and in the soil the sugars and amino acids are supposed to be released by micro-organisms.

A schematic of the polyphenol theory is shown in Figure 4.1 (Stevenson, 1982). Thus the polyphenols can be generated from either lignin or by microbial biosynthesis. Again, this theory explains the persistent nature of the association of some of the nitrogen with humic substances. Acid hydrolysis will release a significant portion of the nitrogen from humic and fulvic acids but the balance appears to be intimately bound-up or associated with the carbon fraction. The potential contribution of microbially synthesized polymers is well recognized. Kang and Felbeck (1965) isolated a material similar to humic acid (soluble in alkali, but insoluble in acid) from the spores of Aspergillus niger. Their chemical tests could detect no difference between it and soil humic acids. Visser (1983) isolated fulvic and humic acids from a culture of an unspecified streptomycete and found that their chemical compositions were similar to aquatic fulvic and humic acids.

Andreyuk and Gordienko (1981) found that dark-coloured compounds recovered from cultures of actinomycetes from the Chromogenes and Niger groups were similar to low molecular-weight humic acids as far as spectral and other data were concerned. Linhares and Martin (1978) found melanin type polymers produced by fungi to be similar to humic materials and also to be resistant to microbial degradation in the soil. However, Schnitzer et al. (1973) concluded that fungal humic-like polymers were not similar to the natural substances. Thus, by chemical oxidation with permanganate, they deduced that the former compounds did not contain significant amounts of phenols and phenolic acids. Obviously, the relevance of the microbial polymers is still a matter for debate.

The mechanisms proposed above for the formation of humic substances may each participate in the humification process at different environmental locations depending on the substrates and the environmental conditions. The relative significance of each reaction and hence the end products formed will vary. Hedges (1978), postulated that the formation of marine humic substances involved predominantly the fourth mechanism, namely the condensation of sugars and amino acids to form melandoins (the dark-brown nitrogenous polymers). This theory may explain the lower aromatic content, i.e. approximately 10 - 14%, noted for marine humic acids by Hatcher et al. (1980).

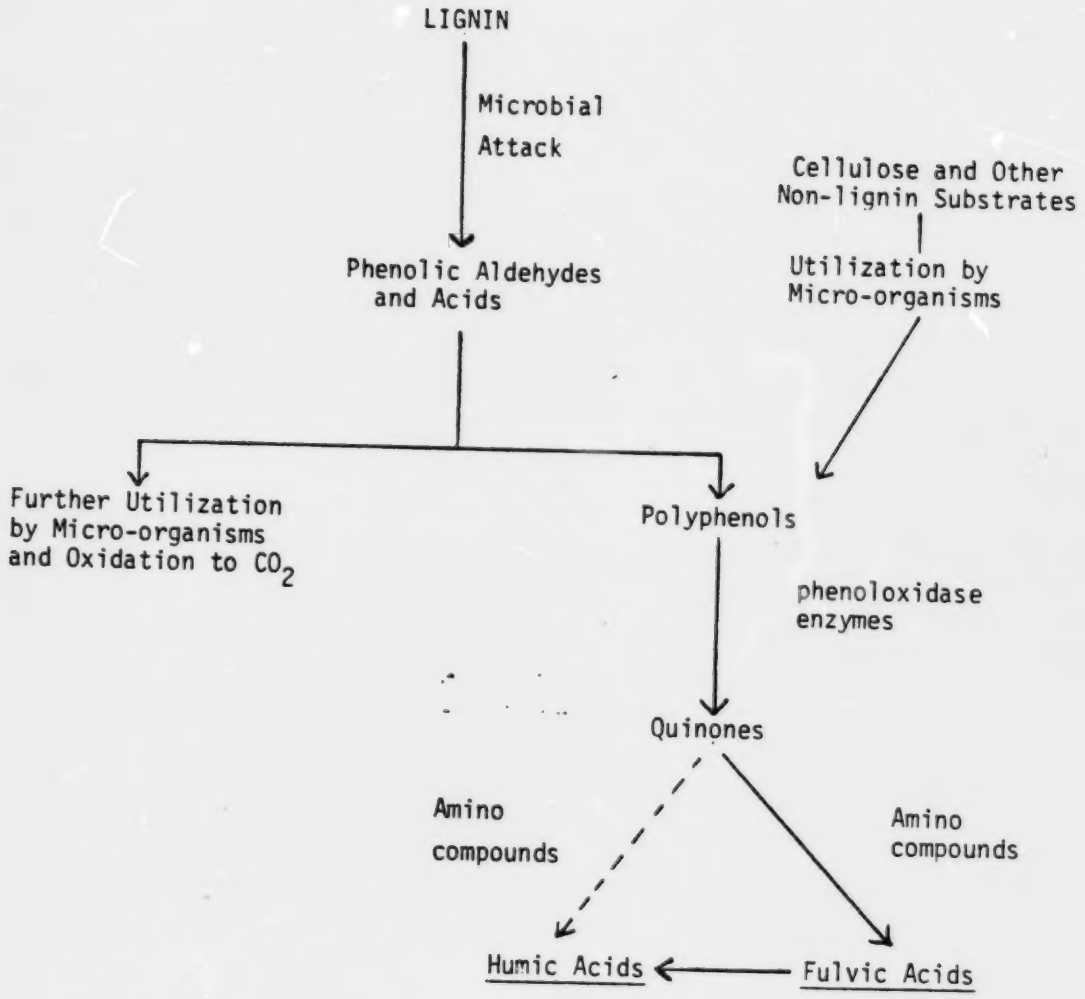
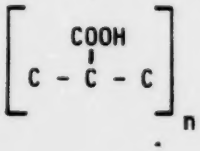


Figure 4.1 Schematic Representation of the Polyphenol Theory of Humus Formation. (Stevenson, 1982)

This brief overview of the current status of knowledge of the structure of fulvic and humic acids as well as how they may be formed in the natural environment emphasizes the level of variation which may be noted between fractions recovered from different sources. This perspective must be borne in mind when interpreting the data on the microbial degradation of humic substances.

Current data (from NMR studies) suggest that the aromatic content of humic and fulvic acids may range from 10 - 40% depending on the source. Polymethylene chains, i.e., aliphatic, may be present in significant amounts and in some instances, the carboxyl content may be high. Wilson et al. (1981) based on NMR data believed that the carboxyl content of freshwater fulvic and humic acids could represent 25% of the fraction, i.e. one in three of the carbon atoms in the structure, could have a carboxyl group attached to it. They suggested the following structural unit.



Such information poses the question of whether one is looking for organisms able to degrade aromatic compounds, aliphatic compounds or highly carboxylated compounds. Possibly all three of these attributes are required.

This brief outline of a biological role in the formation of very stable humic substances and particularly humic acids indicates why it may be very difficult to degrade these compounds biologically. By their nature they help the organisms which produce them to resist biological attack, i.e. they are designed to be resistant to biological decay . Additional data using radiolabelled C and N compounds suggest that the degradation/formation of humic substances in the natural environment is a dynamic process by which the humus content of the soil is maintained in a state of equilibrium (Freytag, 1971; Igel, 1971). Data by Debosz and Golebiowska (1975) appear to support this hypothesis. These authors demonstrated that an initial degradation of soil humic materials took place under certain cultural conditions over a four week period. However, by the end of six weeks, the degradation products appeared to have reformed into humic-like substances.

4.3.3 Kerogens

A fraction related to humic substances which should be reviewed briefly is the kerogen material. Kerogen is defined as "the insoluble organic matter occurring in sedimentary rocks."(Forsman, 1963). The kerogen content of such deposits is estimated to be 0.3%. Compared with humic substances, the kerogens have a significantly higher carbon content (approximately 80%) and a lower oxygen concentration (approximately 10%) (Forsman, 1963). Similarly, the carbon to nitrogen ratio can range from 30 to more than 500 compared with the value of approximately 10 for mature humus. However, the higher ratios appear to be associated with finer particles in a kerogen sample while the lower ratios are found in the larger particles, i.e., there appears to be two different nitrogen-containing components present. Oxidative degradation of kerogens by permanganate yields oxalic acids equivalent to as much as 36% of the carbon oxidized (Forsman, 1963). They could, therefore, be a source of oxalic acid in the Bayer Process. It is not known, however, if they are present in the bauxite ores.

The data from chemical degradation studies led Forsman to conclude that of three possible types of kerogens in rocks, one type is very similar to the humic substances found in "lignins, peats and coals."

Data from vacuum pyrolysis studies applied to humic acids and kerogens indicated that the kerogens yielded high amounts (appoximately 5 times more) of straight chain hydrocarbons than the humic acids. This may indicate a higher aliphatic content within the kerogens (Fukushima, 1982).

No articles describing the microbial attack on kerogens have been identified to the present time.

4.3.4 The Relationship between Lignin and Humic Substances

The assumed close relationship between lignin and humic substances and the extensive information published on the biological degradation of lignin suggest that the question of whether the information from the lignin studies might assist this study should be considered. In particular, the ability of certain fungi to decolorize kraft mill effluents may relate to the topic of this report.

Natural lignins are much more aromatic in nature and have a higher content of methoxyl groups than humus. This is supported by the lower aromaticity being claimed for humic substances using the newer techniques of NMR, etc. Since the humic substances represent an end product of lignin degradation (irrespective of the precise mechanism by which it occurs), it is doubtful if the studies on natural lignins are of direct relevance.

Experimental studies on the use of the fungi such as Tinctoporia sp.(Fukuzumi et al., 1977), Coriolus versicolor (Livernoche et al., 1983) and Phanerochaete chrysosporium (Eaton et al., 1982) to remove colored lignin derivatives present in kraft mill effluents were also investigated. All of these species were effective in reducing the color level of the effluent by factors as high as 90% after 10 days. The organisms are believed to degrade the kraft lignins although the need for a supplemental carbon source for the organisms suggested that the decolorization reaction was a secondary metabolic event (Eaton et al. 1982). The composition of the kraft lignins compared with that of humic substances is important. The colored components in kraft lignin in an effluent such as the first alkaline extract solution after chlorination, contain oxylignin (conjugated double bonds, quinoid structures) and partially chlorinated compounds (Fukuzumi et al., 1977). Such compounds although similar are not directly related to the structures believed to be present in humic substances and therefore the expectation of gaining information from these decolorization studies is considered slight at the present time. However, the mechanisms postulated for the biologically catalyzed degradation of lignin may be of interest when the mechanisms of degradation of humic substances are considered.

4.4 - MICROBIAL ATTACK ON HUMIC SUBSTANCES

In this section of the report, we will review the published data on the ability of bacteria, fungi and actinomycetes to degrade humus or isolated humic fractions.

4.4.1 Bacteria

The bacteria identified as degrading humic substances are listed in Table 4.4. The list is limited and reflects the greater prevalence of fungi and actinomycetes in degradation reactions in the soil.

Kunc et al. (1976) separated fulvic acid into four fractions, namely fulvenic acid, lignofulvonic acid, fulvinic acid and fulvanic acid. The separation method is not available to the Authors of the report but the stated elemental compositions of the fractions are shown in Table 4.5 All preparations contained aromatic residues. Fulvanic acid appeared to be more acidic than the other fractions and this was confirmed by the stronger signal for carbonyl groups (from carboxylic acids) in the IR spectrum. The authors tested the ability of organisms in a soil (Chernozem) to degrade the various fractions. Mixtures (0.2%) of the fulvic acids in soil suspensions were incubated at 28^{o}C and growth of the cells on the fulvic acid as carbon source was monitored either by manometric measurement of oxygen uptake or by quantitation of the release of carbon dioxide. The bacterial growth in the soil suspension was better in the case of the lignofulvonic and fulvenic fractions with carbon mineralization being 48.4 and 79.8% respectively after 10 days' incubation. During the incubation, the numbers of bacteria in the soil increased and the soil organisms also developed an improved ability to oxidize aromatic compounds such as vanillic and p-hydroxybenzoic acids. This suggested that the microbial attack was against aromatic structures in the fulvic acids.

Mishustin and Nikitin (1961) hypothesized that the ability of a Pseudomonas sp. to actively decolorize humic acid (60% loss of colour within 14 days) involved the oxidation of the humates by the enzyme peroxidase.

Table 4.4 Bacterial Utilization of Humic and/or Fulvic Acids

Organism	Substrate	Incubation Period	% Degradation	Comments	Reference
Agrobacterium 1	HA (0.2%)	7 weeks	11.5	HA - Sole source of C and/or N (Czapek Dox broth) HA utilization measured by decrease in optical density.	Mathur & Paul (1967)
Agrobacterium 2	HA (0.2%)	7 weeks	7.5		Mathur & Paul (1967)
Pseudomonas sp.	HA (0.2%)	7 weeks	15.4		Mathur & Paul (1967)
Bacillus sp.	HA (0.2%)	7 weeks	5.7		Mathur & Paul (1967)
Mixed soil organisms	FA-1 (0.2%)	10 days	48.4	Soil culture - FA utilization measured by CO_2 release.	Kunc *et al.* (1976)
Mixed soil organisms	FA-2 (0.2%)	10 days	79.8		Kunc *et al.* (1976)
Pseudomonas sp.(V3)	FA-1 (0.2%)	6 hours	ND	Pure cultures FA utilization followed by CO_2 - release	Kunc *et al.* (1976)
Pseodomonas sp.(V3)	FA-2 (0.2%)	6 hours	ND		Kunc *et al.* (1976)
Pseudomonas sp.(K 37)	FA-1 (0.2%)	6 hours	ND		Kunc *et al.* (1976)
Pseuodmonas sp.(K 37)	FA-2 (0.2%)	6 hours	ND		Kunc *et al.* (1976)
Escherichia coli (K 12)	FA-1 (0.2%)	6 hours	ND		Kunc *et al.* (1976)
Escherichia coli (K 12)	FA-2 (0.2%)	6 hours	ND		Kunc *et al.* (1976)
Pseudomonas putida	FA		NA		Rifai & Bertru (1990)
Pseudomonas fluorescens	HA		NA		Gordienko & Kunz (1984)
Bacillus megaterium	HA		NA		Gordienko & Kunz (1984)
Mycobacterium sp.	HA		NA		Gordienko & Kunz (1984)

HA = Humic Acid ; FA = Fulvic Acid ; ND = No Degradation NA = Not available
FA-1 = Lignofulvonic Acid ; FA-2 = Fulvenic Acid

Mathur and Paul (1967) found that of four bacterial species isolated from soils, a Pseudomonas sp. utilized a humic acid in Czapek Dox broth as its sole source of carbon. After four weeks the % utilization of the humic acid was approximately 15% based on optical density measurements. Two Agrobacterium spp. and a Bacillus sp. only achieved a 5 - 11% degradation of the humate. The Bacillus sp. also utilized the humic acid as a sole source of carbon but the Agrobacterium spp. utilized the humic acid as a source of both carbon and nitrogen.

TABLE 4.5

VALUES FOR C AND N IN FULVIC ACID FRACTIONS

(Kunc et al., 1976)

Fulvic Acid Fraction	Elemental Concentration (%)		C:N	pH*
	C	N		
Fulvenic Acid	28.5	14.0	2.0	5.85
Ligno Fulvonic Acid	41.1	9.5	4.3	5.60
Fulvinic Acid	47.0	10.0	4.7	6.05
Fulvanic Acid	52.8	4.0	13.2	3.50

* pH value of 0.1% (w/v) aqueous solution

4.4.2 Fungi

Data on the decomposition of humic substances by fungi are summarized in Table 4.6 Some qualitative data to be discussed hereafter are not listed in the Table.

Early studies on the microbial degradation of humic substances (Flaig and Schmidt, 1957) had claimed that Penicillium spp. (including P. frequentans, P.spinulosum, P.lividum, P.roseo-purpureum), could not utilize humic acid as their sole carbon source. Nitrogen in the humic preparation could be used to a small extent. Burges and Latter (1960) isolated two fungal species belonging to the Spicaria and Polystictus genera. Using soil percolation columns and a suitable medium they extracted 33.3% of the humic acids with the Spicaria sp. and 42.8% with the Polystictus over a period of 8 weeks. In a comparison test using 0.12% humic acid in agar medium the organisms digested 17% of the humic acid in the case of Spicaria and 64% for Polystictus after 6 weeks at 25^{o}C. The medium used contained 2% sucrose, so it still did not verify that the organisms would utilize the humic acid as their sole source of carbon.

In a subsequent series of tests, Latter and Burges (1960) noted that the Spicaria sp. was in fact a strain of Penicillium luteum. They confirmed that the Polystictus in the presence of a C and N source achieved 72% decomposition of a 0.06% humic acid solution (within 6 days). A total of 81% decolorization was measured but 9% of this value was attributed to chemisorption of the humic acid on to the mycelium.

Mathur and Paul (1967) compared the ability of Penicillium frequentans (two strains), Aspergillus versicolor, a Fusarium sp., a Cephalosporium sp., a Cunninghamella sp. and a Penicillum sp. strain 895 to utilize humic acid as sole source of carbon or nitrogen. One of the strains of Penicillium frequentans could effectively use the humic acid as either a sole source of C or N. Under more optimized conditions the Penicillium sp. strain 895 could degrade 35% of 0.02% (w/v) humic acid in a culture medium within a 7 week period.

Table 4.6 Utilization of Humic and/or Fulvic Acids by Fungi

Organism	Substrate	Incubation Period	% Degradation	Comments	Reference
Penicillium frequentars 1	HA (0.2%)	4 weeks	17.3	Czapek Dox broth.	Mathur & Paul (1967)
Penicillium frequentans 2	HA (0.2%)	4 weeks	7.7	Humic acids added as sole	Mathur & Paul (1967)
Aspergillus versicolor	HA (0.2%)	4 weeks	5.7	source of C and N.	Mathur & Paul (1967)
Fusarium sp.	HA (0.2%)	4 weeks	5.7	Photometric measurement.	Mathur & Paul (1967)
Cephalosporium sp.	HA (0.2%)	4 weeks	7.7		Mathur & Paul (1967)
Cunninghamella sp.	HA (0.2%)	4 weeks	7.7		Mathur & Paul (1967)
Penicillium 895	HA (0.2%)	4 weeks	7.7		Mathur & Paul (1967)
Penicillium frequentans	HA (0.2%)	7 weeks	35.	Czapek Dox broth HA as sole source of C & N. Replacement culture - shaken. Photometric measurement.	Mathur & Paul (1967)
Mixed soil population	FA (0.1%)		ND	No degradation-culture darken	
Poria subacida 17780	FA (0.05%)	24 days	45.3	Czapek Dox broth + Yeast Extr Static culture. Photometric Measurement (Loss of colour)	Mathur (1969)
Poria subacida 17780	FA (0.05%)	48 hours	66.7	As above except replacement culture - no shaking.	Mathur (1969)
Spicaria sp.	HA (0.12%)	8 weeks	33.3	Soil percolation column.	Burges & Latter (1960)
Polystictus sp.	HA (0.12%)	8 weeks	42.8		Burges & Latter (1960)
Marasmius oreades	HA (0.05%)	4 weeks	51.	Czapek Dox broth. HA/FA as sole source of C & N. Replacement/static culture. Photometric measurement.	Mathur (1970)
Marasmius oreades	FA (0.05%)	4 weeks	18.	As above	Mathur (1970)
Fusarium solani	HA (0.1%) (Manure)	40 days	40.	Mineral salts + C & N Suppl. Photometric measurement.	Khandelwal & Gaur (1980)
Fusarium solani	HA (0.1%) (Soil)	40 days	18.	As above	Khandelwal & Gaur (1980)
Pen. roseopurpureum	HA (0.1%)	40 days	24.4	As above) Manure HA	Khandelwal & Gaur (1980)
Aspergillus fumigatus	HA (0.1%)	40 days	31.1	As above)	Khandelwal & Gaur (1980)

HA = Humic Acid FA = Fulvic Acid ND = Not Determined

Hurst et al. (1962) surveyed 29 strains of fungi for their ability to decolorize humic acid and also for production of an enzyme able to reduce m-hydroxybenzoic acid. This latter test was included because in preliminary tests of the action of the fungi on simple monohydroxybenzoic acids there was no indication of fission of the aromatic ring. Instead, the product was m-hydroxybenzyl alcohol, i.e. the reduced product from the carboxylic acid. They obtained a positive correlation between the ability to decolorize the humic acid and the presence of reducing ability on the aromatic carboxyl. The active species were Polystictus versicolor (L), Hypholoma fasciculare (3 strains - all positive), Trametes suaveolens (L), Trametes rubescens, Polyporus brumales, Polyporus arcularius, Polyporus resinosus, Polyporus schweinitzii and Ustulina zonata. Of interest, was the fact that whereas one strain of T.suaveolens (L) (Strain A) degraded the humic acid, a second strain (Strain B) gave a negative reaction. Thus the ability to decolorize the humic acid was not only species dependent but also dependent on the strain.

Based on their results, Hurst et al. (1962) suggested that the initial reaction with the humic acid involved the reduction of the carboxylic group(s) of the humic acid. However, in view of the aerobic growth of the organisms, the reductive capacity was considered to be a secondary reaction of the aerobic metabolism of the other carbon source in the medium, i.e., 0.5% (w/v) glucose. No evidence was presented of whether or not the organisms could utilize the humic acid as sole carbon source. The nitrogen source in the medium also influenced the efficiency of the decolorization reaction. There were no quantitative data in this study.

Dubovska and Macor (1978) compared the ability of 14 fungal species to utilize fulvic or humic acids in a culture medium containing 1.5% glucose and 0.15% sodium nitrate. Humic/fulvic acid concentrations were 0.05%. The most active species against fulvic acid were Aspergillus versicolor, Actinomucor repens, Penicillium lilacinum, Absidia glauca, Trichoderma koningi, Curvularia subulata and Penicillium decumbens. Similarly, the most effective species against humic acids were Penicillium citrinum, Penicillium funiculosum and Aspergillus sydowi. These conclusions were based on the relative yields of mycelium (g/unit volume).

This approach was used further by Macor (1979)to monitor the growth of the fungi on fulvic acids (separated into 4 factors) and humic acids fractions (separated into 2 fractions). Fourteen (14) fungi were evaluated including all of the above species (Dubovska and Macor, 1978) as well as Curvularia lunata, Cephalosporium curtipes, Penicillium terrestre and Penicillium expansum. The experimental conditions used were stationary culture at 25°C for 60 days. The humic/fulvic fractions were included in the medium (Czapek Dox broth) at 0.05% (w/v) and to test the ability of the organisms to utilize the humic materials as sole C or sole N source, the nitrogen or carbon sources in the Czapek Dox broth were omitted in appropriate test cultures.

This was done for each organism. The mean data from comparing the weight of mycelium from each test with the appropriate control for that test gave an indication of growth patterns on the humic substances. Based on the numbers of tests, generalizations are possible. It appeared that the organisms utilized the humic acids less effectively than the fulvic acids possibly because the latter group are smaller molecules and less condensed than the humic acids. The data suggested that the ability to use the various fractions was very much species dependent and that certain organisms appeared to preferentially use either the carbon or nitrogen component of the humus fractions. The humus was isolated from a peat soil and had a C:N ratio for the 6 fractions noted above ranging from 15 - 72, i.e. relatively immature humic materials were present.

All of the previous tests used relatively low concentrations of humic fractions. Mathur (1969) using Trichoderma viride, Penicillium frequentans, and Aspergillus fumigatus (each isolated by enrichment from soils), as well as a white rot fungus Poria subacida 17780 found that 0.1% (w/v) fulvic acids in liquid cultures inhibited completely the growth of all four organisms. At concentrations of 0.05% fulvic acid, all of the soil isolates did not utilize the fulvic acid in liquid cultures (either static or replacement, with or without shaking at 28°C). Instead, the cultures

darkened and tests indicated that conversion of fulvic acid to humic acids was occurring in these cultures. White rot fungus P. subacida used fulvic acid (0.05%) as sole source of carbon. Aeration was inhibitory to the fulvic acid utilization and in aerated cultures the intensity of the colour increased. However, it did not appear to be humic acids that were being formed as noted in the case of the soil isolates. P. subacida in static, replacement cultures* (+ nutrients) rapidly decolorized 0.05% fulvic acid to the extent of 67% in 48 hours. The enzyme phenol oxidase was present in the culture filtrates of P. subacida but did not appear to be involved in the breakdown of the fulvic acid, i.e. in replacement static culture the enzyme activity did not appear in the cultures until after the decolorization had reached the level of 67%. The replacement culture technique helps to demonstrate that the loss of colour is not due to adsorption of fulvic acid on to mycelium but rather by degradation.

The loss of fulvic acid as measured by decolorization was confirmed by precipitation techniques. The degradation appeared to be enzymatically catalyzed. Thus, particulate and soluble extracts from cells of P. subacida could catalyze the reaction to the extent of 18% loss of color of fulvic acid from solution (0.025%) in 200 min. at 28^{o}C. Heating destroyed the ability of these preparations to decolorize the fulvic acid solutions and this is very indicative of a biological agent (enzyme) being involved.

Mathur (1970) noted that P. subacida did not utilize humic acids. Penicillium frequentans could utilize humic acid but not fulvic acid. Marasmius oreades (Bolt)Fr., the fungus of fairy ring mushroom, utilized both fulvic acid and humic acid in Czapek Dox broth in stationary cultures (44% and

* FOOTNOTE: In a replacement culture, fungal mycelium is grown in culture medium in presence of fulvic acid, the mycelial mat is recovered, washed, soaked in fulvic acid solution to saturate adsorption sites on the mycelium, washed again with sterile water and then transferred to culture medium containing fulvic acid as the sole source of carbon.

15% respectively after 1 day in replacement culture). The reactions were inhibited by shaking. This inhibition by aeration, similar to that noted with fulvic acid decolorization by P. subacida, was attributed to the activity of phenol oxidases. It was noted that the utilization of both humic and fulvic acid by M. oreades was quite rapid and during the first day, approximately 90% of the total loss after 1 week had been achieved.

Khandelwal and Gaur (1980) screened six fungi for their ability to degrade a humic acid. They found that three species could effectively degrade this substrate as sole C and N source, but that the presence of glucose and a nitrogen source (ammonium sulphate) in the medium enhanced the organisms' abilities to cause decomposition. The respective degradation efficiencies in a mineral medium containing 0.1% humic acid were, at the end of 40 days' incubation at 30°C, 31% for Aspergillus fumigatus (F7), 40% for Fusarium solani (F4), and 24% for Penicillium roseopurpureum (F5). These tests were performed with a manure humic acid (i.e. relatively immature). Fusarium solani was also effective against an older soil humic acid (18% after 40 days), but only if carbon and nitrogen sources were available.

This benefit from having supplemental carbon and nitrogen sources in a culture medium is quite common and may relate to the observations of Ruocco and Barton (1978). They demonstrated that Aspergillus niger mycelium, if suspended in a solution of humic acids at 25°C, would take up the humic acid and, more particularly, that the uptake was in part dependent on the cells' metabolic processes. Thus, the addition of metabolic inhibitors such as 2,4-dinitrophenol or sodium azide, which inhibit key enzymes in the respiratory cycle of the organism, prevented the uptake of humic acid by A. niger. The enzyme inhibitors did not totally inhibit the uptake of the humic acid from solution as some non-specific adsorption was also noted, even with lysed (dead) cells. Such non-specific adsorption led some investigators to question the validity of loss of colour as an indicator of humic or fulvic acid degradation (Mishustin and Nikitin, 1961). The efficiency of uptake was also markedly dependent on the pH with an optimum uptake occurring between pH 3-4. Temperature also affected the energy-dependent uptake of humic acid with the optimum capacity occurring at 30°C.

There appears to be strong evidence that the degradation of humic substances by fungi is dependent for optimum effect on the presence of alternate energy sources (i.e. carbon nutrients) in the culture medium. In some cases, the presence of a nitrogen supplement is also beneficial. Thus the degradation of humic substances is a secondary reaction of the cells' metabolic pathways. The significant variations between the abilities of different species and different strains of the same species to degrade humic substances may reflect the abilities of the respective organisms to utilize the carbon and nitrogen compounds i.e. biopolymers, entrapped within the humic substances. This will be influenced by the individual purities (i.e., nitrogen contents) of the humic fractions used by the different researchers. In most instances, such information is not documented.

The role for a reducing enzyme in the decolorization reactions (noted by Hurst et al. 1962) was also apparently a secondary reaction and dependent on the presence of alternate energy sources.

4.4.3 Actinomycetes

Information on the degradation of humic substances by actinomycetes is summarized in Tables 4.7A and 4.7B It should be noted that bacteria of the coryneform group of organisms, e.g. Arthrobacter sp., have been included in this unique collection of filamentous bacteria. Such a division is an arbitrary one.

Actinomycetes are classified as autochthonous soil micro-organisms considered to be indigenous to the soil (Kuster, 1979). As such, selected species have been implicated in the decomposition of lignin and the reader is directed to separate reviews (Crawford and Crawford, 1984; Amer and Drew, 1980).

Table 4.7A Utilization of Humic and/or Fulvic Acids by Actinomycetes

Organism	Substrate	Incubation Period	% Degradation	Comments	Reference
Streptomyces antibioticus	HA (0.2%)	46 days	17.	Minerals salts + sand.	Monib et al.(1981)
S. noursei	HA (0.2%)	46 days	17.	Humic acid as sole source of C & N.	
S. parvullus	HA (0.2%)	46 days	20.	Utilization measured by CO_2 evolution.	
S. reticulus	HA (0.2%)	46 days	14.	Moisture - 60%	
S. aureofaciens	HA (0.2%)	46 days	19.		
S. ramulosus	HA (0.2%)	46 days	15.		
S. galilaeus	HA (0.2%)	46 days	11.		
S. albogrisiolus	HA (0.2%)	46 days	16.		
S. viridogenes	HA (0.2%)	46 days	13.		
S. hygroscopicus	HA (0.2%)	46 days	14.		
S. venezulae	HA (0.2%)	46 days	13.		
S. olivaceus	HA (0.2%)	46 days	19.		
S. prasimus	HA (0.2%)	46 days	12.		
S. griseus	HA (0.2%)	46 days	17.		
S. griseoflavus	HA (0.2%)	46 days	17.		
S. viridochromogenes	HA (0.25)	46 days	13.		
S. michiganensis	HA (0.2%)	46 days	15.		
S. rubrireticuli	HA (0.2%)	46 days	12.		
S. niveoruber	HA (0.2%)	46 days	11.		
S. phaeochromogenes	HA (0.2%)	46 days	12.		
S. fradiae	HA (0.2%)	46 days	12.		
S. purpurescens	HA (0.2%)	46 days	15.		
S. netropsis	HA (0.2%)	46 days	19.		
S. tendae	HA (0.2%)	46 days	12.		

Table 4.7B Utilization of Humic and/or Fulvic Acids by Actinomycetes (Continued)

Organism	Substrate	Incubation Period	% Degradation	Comments	Reference
Streptomyces violaceoniger	HA (0.2%)	46 days	15.	Mineral salts + sand.	Monib et al. (1981)
S. erythraeus	HA (0.2%)	46 days	17.	Humic acid as sole source of C & N. Utilization measured by CO_2 evolution.	Monib et al. (1981)
S. lavendulae	HA (0.2%)	46 days	21.		Monib et al. (1981)
S. fulvissimus	HA (0.2%)	46 days	17.		Monib et al. (1981)
Streptomyces sp. (A 31)	HA (0.1%)	40 days	20.0	Mineral salts supplemented with C & N. Tests using manure humic acid. Photometric measurement. (loss of colour).	Khandelwal & Gaur (1980)
Streptomyces sp. (A 32)	HA (0.1%)	40 days	24.4		Khandelwal & Gaur (1980)
S. candidus (A 11)	HA (0.1%)	40 days	47.8		Khandelwal & Gaur (1980)
S. candidus (A 12)	HA (0.1%)	40 days	44.4		Khandelwal & Gaur (1980)
S. garyphalus	HA (0.1%)	40 days	24.4		Khandelwal & Gaur (1980)
S. rubescens	HA (0.1%)	40 days	28.9		Khandelwal & Gaur (1980)
S. violaceoruber (A 41)	HA (0.1%)	40 days	24.4		Khandelwal& Gaur (1980)
S. violaceoruber (A 42)	HA (0.1%)	40 days	31.1		Khandelwal & Gaur (1980)
S. candidus	HA (0.1%)	40 days	26.0	As above except soil HA.	Khandelwal & Gaur (1980)
Proactinomyces citreus	HA	150 days	ND	Decomposition of straw.	Ivanova (1979)
Proactinomyces corallinus	HA	150 days	ND		Ivanova (1979)
Micromonosporaceae family	HA	150 days	ND		Ivanova (1979)
Mixed actinomycetes (30)	HA	NS	ND	Czapek Dox broth. HA sole source of C & N.	Mathur & Paul (1967)
Arthrobacter sp.	HA	4 weeks	11.5		Mathur & Paul (1967)
Arthrobacter globiformis	FA	NS	ND	Stimulated by benzoate & lactate.	Rifai & Bertru (1980)

HA = Humic Acid ; FA = Fulvic Acid ; ND = Not Determined

However, the ability of selected species of actinomycetes to utilize humic acid (isolated from farmyard manure) as a sole source of carbon was demonstrated (Khandelwal and Gaur, 1980). Nine species grew in mineral salts medium containing the humic acid (0.1%) as sole carbon source, sole nitrogen source or sole C & N source. Over an incubation period of 40 days at 30°C, these organisms could utilize the humate as either sole carbon or nitrogen source. However, when the medium was supplemented with glucose (0.25%) and ammonium sulphate (0.1%) as additional C and N sources, the utilization of humate (as indicated by photometric measurements) was increased significantly, i.e. by a factor of 2-3. Of the nine species (see Table VIIA) only four of these could decolorize the humic acid in a gel medium, and, in the same test, a fungus, Fusarium solani, was a much more efficient decolorizing agent. This indicates that the utilization of humate need not necessarily involve decolorization. In some situations, the actinomycetes may actually bring about the conversion of low molecular-weight fragments to humic compounds. One strain (Streptomyces candidus) was tested by Khandelwal and Gaur for its ability to degrade a soil humic acid, i.e. a more mature humate than the manure-derived material. The organism utilized the soil humic acid as both a sole C and N source and supplementing the medium with glucose and ammonium sulphate gave only a slight improvement in the utilization of humate substance.

Monib et al. (1981) compared the humic acid degrading abilities of 28 Streptomyces species. The test medium contained 0.2% (v/v) dried humic acids (0.117% C) as sole source of carbon. The production of mycelium, change in organic carbon content of culture filtrates and the optical densities of the culture filtrates were monitored over a period of 4 months (120 days). In coincident tests, the humic substance was mixed with sterile sand and inoculated with each organism. Under controlled conditions, the ability of each organism to utilize the humic acid as sole carbon source was monitored by the release of carbon dioxide, over a period of 46 days at 30°C.

All of the Streptomyces species could utilize the humic acid as sole C source and, based on the release of CO_2, the utilization of humic acid by the different species over 46 days ranged from a low of 11% to a high of 21%. A significant portion (25-66%, depending on the organism) of this carbon utilization occurred within the first 3 days, due most probably to the utilization of the more easily degraded carbohydrates, protein, etc., present as contaminants in the humic acid.

In the liquid culture, the reduction in organic carbon content was taken as the more reliable monitor. The authors calculated that the consumption of humic acid was 37-59% of the original amount present (dependent on the individual organism). The authors noted that the resistance of humic acids to chemical oxidation may have biased the organic carbon determinations. However, the use of the photometric procedure was also prone to error. Generally, the photometric data indicated that the organisms did utilize the humic acids but the carbon consumption after four months was much lower (19-34%) than in the case of the organic carbon analyses. In some cultures, rapid; significant increases or decreases in optical density occurred. This was believed to indicate the fact that certain Streptomyces species will synthesize phenolic compounds and give rise to new, dark-coloured humic related substances. This ability of streptomycetes to synthesize these dark-coloured polymers from simple organic substances has been well documented (Martin and Haider, 1968; Ivanova, 1979; Stevenson, 1982), and has been discussed briefly in an earlier section of this report.

Debosz and Golebiowska (1975) also found that the ability of pure cultures of Streptomyces species to utilize humic acids (0.1%) was a species dependent. However, in aerated cultures, the synthesis of humic substances appeared to be favoured over the decomposition reaction. With a mixed culture of soil organisms, there was an initial degradation (i.e. 80-100% in some instances) of humic acids within the first 4 weeks, depending on the supplements added (e.g. glucose, mannite, etc.). However, after 6 weeks of

incubation, there was evidence of a significant resynthesis of humic substances within these same cultures. This indicates the dynamic nature of the degradation/synthesis reactions considered by some researchers to represent the status of humic substances in the soil. Under natural conditions, the humus contents of soils are believed to reach a state of equilibrium. It is of interest to note that the addition of only glucose as supplementary carbon source for a soil inoculation, appeared to enhance the degradation of both soil and lignite humic acids by the mixed culture of organisms (i.e. 50-100%). However, the addition of glucose and a nitrogen supplement appeared to favour synthesis of the humic substances. These latter tests did not indicate whether bacteria, fungi, or actinomycetes were predominant.

Ivanova (1979) also noted the ability of actinomycetes to both degrade and resynthesize humic substances. His data supported the belief that the autochthonous soil microflora, which are believed to be responsible for the degradation or mineralization of humic substances in the soil, include both Proactinomyces and Micromonosporaceae. Again, the presence of nitrogen accelerated the degradation of organic matter (straw) and the rate of formation of humic substances. The precise species present at earlier stages of the degradation process were not identified.

Arthrobacter globiformis could degrade fulvic acid (Rifai and Bertru, 1980). The presence of benzoate and lactate appeared to stimulate the degradation process, possibly due to their enhancement of the production of extracellular enzymes able to hydrolyze aromatic groups.

Mathur and Paul (1967) attempted to confirm the ability of soil actinomycetes to degrade humic acid. They tested 30 actinomycetes from soils but found that none of them would effectively utilize humic acid. They did observe that an Arthrobacter sp.could utilize fulvic acid in Czapek Dox broth (carbon and nitrogen components omitted) to the extent of 11.5% based on loss of colour.

4.4.4 Oxalate-utilizing Micro-organisms

As described earlier, oxalate and other aliphatic carboxylic acids are products of the chemical degradation/oxidation of humic substances during mineral processing operations such as the Bayer process applied to bauxite ore.

Oxalate-utilizing organisms, both aerobic and anaerobic have been isolated or identified in soils, sediments and the rumens of herbivores. They include various pseudomonads (Jakoby and Bhat, 1958) including Ps. oxaliticus whose metabolic processes have been extensively studied (Blackmore et al., 1968; Dijkhuizen et al., 1977), Bacillus extorquens and Vibrio oxaliticus (Bhat and Barker, 1948), actinomycetes such as Nocardia sp. (McClung, 1954) and Streptomyces sp. (Khambata and Bhat, 1954), Alcaligenes sp. (Chandra and Shethna, 1975) and Spirillum sp. (Van der Kooij and Hijnen, 1984). Anaerobic organisms have included Clostridium sp. (Bhat, 1966) and unidentified cultures from sediments (Smith and Oremland, 1983) and ruminants (Allison and Cook, 1981). Similarly, fungi such as Aspergillus niger (Emiliani and Bekes, 1964) and Myrothecium verrucaria (Lillehoj and Smith, 1965) have been identified to degrade oxalate.

Based on the composition of the Bayer process liquor, it is difficult to envisage any of the above organisms being used to remove oxalic and the other carboxylic acids from the actual process liquor. To do so would require extensive neutralization of the liquor which would not be acceptable for economic reasons. They will not, therefore, be considered further at the present time.

4.4.5 Effect of Other Soil/Ore Components

All of the above studies have used relatively clean solutions or sands. Humic substances such as fulvic acids and humic acids tend to adsorb on to the surfaces of clay and other silicate minerals. When this happens, the rate of microbial degradation of the humic materials is significantly reduced (Schnitzer et al., 1980; Varadachari et al., 1984). It is not known to what extent the other components of the bauxite ores might interfere in the rate of degradation of humic substances but it could be an important factor.

4.5 CONCLUSIONS AND RECOMMENDATIONS

4.5.1 General

A literature review has been conducted on the microbial degradation of humic substances, fractions isolated from humic substances, and compounds derived from the chemical degradation of humic substances during the processing of ores. In the last named group, the troublesome products are predominantly aliphatic carboxylic acids such as oxalic acid. The feasibility of removing these degradation products from process liquors is considered to be poor.

4.5.2 Biological Degradation of Humic Substances

The data described earlier indicate that humic degrading micro-organisms are present in the natural environment of various soils. Organisms known to degrade humic substances include bacteria, fungi and actinomycetes. However, the rates of degradation of the humic fractions such as humic acid or fulvic acid are generally slow. Thus, incubation periods of 4 - 12 weeks may be required to achieve destruction of the humic substrates with efficiencies in the order of generally 20 - 50%.

The studies reported herein have, in many instances, used isolated organisms in liquid cultures. It is recognized that humic substances in the natural environment tend to adsorb on to the surfaces of silicate and other particles. This adsorption is considered to render the fractions such as the fulvic and humic acids more resistant to microbial degradation. This factor must be investigated further.

The fractions, which have been more extensively studied, are the lower molecular weight fractions from natural humus. The higher molecular weight fraction (i.e. humin), which may amount to more than 50% of the original humus, will be even more refractory. Data on its degradation are scarce. Compounds such as the kerogens may also be present in bauxite ores and, if so, will prove a formidable challenge to the micro-organisms. It is not known if the micro-organisms will degrade these kerogens.

The ability of organisms to degrade either humic acid or fulvic acid is apparently species dependent. Similarly, organisms able to degrade both of these compounds tend to be more effective against the fulvic acid fraction. This is thought to be due to the fulvic acid being of lower molecular weight and less condensed. These varying abilities obviously reflect the differing metabolic capabilities of the various species of organism. Different strains of the same species of organism will have widely differing abilities to degrade the target humic compounds.

In many cases, the organism will utilize the humic substance as its sole source of either carbon or nitrogen. However, in most tests, the addition of carbon and nitrogen supplements was beneficial to the degradative process and apparent conversion efficiencies were normally higher in the presence of these supplements. This was particularly true in the case of the fungi with the carbon as alternate energy source. Thus, in many fungi, the degradation of humic and fulvic acids appears to be a secondary metabolic process and may involve enzymatic action on specific carboxyl groups of the humic materials. This explains the apparent need for these supplements. Energy-metabolizing processes also appear to be involved in the mechanism by which humic acid is transported into the fungal cells.

Actinomycetes are well known for their degradative ability. However, for the current purpose care may be required in investigating them. In addition to bringing about the degradation or mineralization of humic substances, they are also believed to be involved in the re-synthesis of new humic substances from low molecular organic compounds. Fungi can also form the melanin-type pigments similar in composition to humic substances. It is obviously a complex situation, and at the present time the data are inadequate to make strong recommendations as to the more promising organisms for possible study.

The solution may lie in the use of mixed cultures of the specific organisms referred to in the text. However, in the natural environment, the tendency will be for the indigenous micro-organisms to re-establish dominance unless conditions can be maintained favourable at all times for the added organisms. This will require careful assessment of many factors including the chemical composition of the humic substances in a bauxite ore, the candidate organisms and the cultural conditions to optimize destruction of the humics etc. It must be regarded as a relatively large undertaking.

4.5.3 Recommendations

Based on available data, the following recommendations are made for preliminary evaluation of the technical feasibility of applying the use of micro-organisms to the degradation of humic substances in bauxite ores:

- The humic materials present in the bauxite ores need to be more precisely identified with each ore being regarded as an individual entity. This characterization should include tests to attempt to identify the fractions most responsible for the organics in the Bayer process liquor. If humins and kerogen-type materials are present in significant amounts, the complexity of the task will be much greater.

- Based on the information gained on the composition of the humic substances in the bauxite ores, attempts should be made to relate the biological data to actual feed pretreatment options.

- At present, the application of biological processes for humic destruction to process related industries is still to be exploited.

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5 - STAGE 3 - CRITICAL ASSESSMENT OF THE STATE-OF-THE-ART

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5.1 - Scope of Work

The scope of work for Stage 3 which is presented here is as follows

(a) Identify specific candidate processes and substitutes/ solutions. At the outset of this critical evaluation, mineral processing operations identified in Stage 1 as being subject to problems due to humic substances or their by- products will be highlighted. These processes may potentially benefit most from a biological solution.

(b) Define the requirements for effective biological destruction methods. It is anticipated that the level of information available on potential processes will be predominantly derived from laboratory studies. Water purification methods at large scale operations may indicate potential routes. These methodologies tend to concentrate on removal of humic substances by adsorption/precipitation rather than biological destruction of the compounds. The economic feasibility of such approaches for mineral processing operations may be poor, but newer technologies using biological regeneration of activated carbon adsorbents may offer attractive features.

In this section, it will be important to define the particular problems that are characteristic of the mineral processing circuits, such as the adsorption of organic substances onto clay/ silicate particles; the chemical composition of the feedstocks; process limitations in handling micro-organisms in slurries; nutrient requirements: volumes of solutions to be handled; environmental factors (temperature, pH, etc): residence times relative to existing chemical procedures.

(c) Compare the information derived in Stages 1 and 2 with the requirements of the stated processes and document the limitations in existing knowledge which prevents or constrains the ready application of biological destruction of organics in mineral processing circuits including

- reaction rates
- relative conversion efficiencies
- toxic factors
- degradation of humic substances adsorbed onto clay/ore particles
- metabolic by-products liable to cause problems in mineral processing circuits
- biological contamination problems
- scale up problems
- economic factors
- nutrient requirements
- effects of temperature, moisture, etc.

(d) Prepare a draft final report for the study.

(e) Review the draft final report with CANMET personnel.

(f) Prepare and submit the final study report including an executive summary in French and English.

5.2 - Candidate Processes

In general, detailed research and development studies on the effect of humic acids in mineral processing operations have not been undertaken. Apart from some isolated information in the aluminum and uranium industries, data on this subject is largely unavailable.

The discussions held with mining companies and the review of of literature obtained during the literature search phase of this study have confirmed that these organic substances do, indeed, produce adverse effects in various unit operations such as

- flotation
- solid/liquid separation
- ion exchange
- solvent extraction.

The humic substances which cause these effects appear to be present in either the ore itself or the process water used during the plant operation.

The latter source gives rise to problems on a seasonal basis, whereas the former produces effects which are constant throughout the year.

The discussion below is restricted to the industries in which data have been, to some extent, obtained.

5.2.1 - Aluminum

The presence of organic compounds in the Bayer Process causes problems during the crystallization of pregnant liquor and, subsequently, in the settling of fine aluminum hydrate crystals during classification. Such operating problems are encountered throughout the aluminum industry, as reported by companies such as Alcan, Kaiser, Indian Aluminum and Dorr-Oliver, and are considered serious.

(a) Background

Organic carbon from 0.1 to 0.6% by weight, as humic, cellulose and protein have been found in bauxite ores. At Alcan, in the Bayer Process, the bauxites with the higher carbon content create problems, as under the conditions of 150-250°C and pH14, the humic acids break down as follows

Bauxite Humic Acids

↓

High Molecular Weight

Humic Fraction

↓

Intermediate Molecular Weight

Benzene Carbolic Acids

Phenolic Acids

Hydroxy Aliphatics

↓

Low Molecular Weight

Formic Acid

Acetic Acid

Oxalic Acid

Succinic Acid

↓

Lactic Acid

Glycolic Acid

↓

Carbonate

In the breakdown, 50% of the carbon ends up in the low molecular weight fraction and 25% in both the high and intermediate molecular weight fractions. Sodium oxalate produced by the breakdown gradually rises in concentration in the the Bayer liquor to an equilibrium-saturation level. Further saturation causes sodium

oxalate crystallization, thus creating problems, such as poor classification-settling of fine Bayer seeds and excessive scaling in process lines and tanks. At present, it is necessary to evaporate part of the solution that is recycled to the bauxite digestion system and then to recover the solids by centrifuging. This technique also removes the high molecular weight fraction and the sodium carbonate. However, the operation is expensive and creates a loss of sodium. The centrifuge cake is difficult to dispose of as it only contains 40% solids and the associated solution has a sodium hydroxide concentration of 450 g/l.

At present, the selling price for aluminum is poor and so any process to overcome this problem must be inexpensive. The only potential cost savings are due to reductions in sodium loss, evaporation requirements and centrifuge cake disposal.

(b) Conventional Processes

Several processes have been considered to treat Bayer liquor and remove the problems caused by the degradation products of humic acids including sodium oxalate.

A number of processes have been tested on a laboratory or mini-plant scale, with varying degrees of success. These include

- high pressure-high temperature oxidation under air and/or oxygen
- electrolytic oxidation
- use of powerful oxidizing agents (peroxides)
- adsorption on activated carbon, activated alumina, fine hydrate and ion exchange resins
- seed washing followed by the precipitation of CaC_2O_4
- precipitation of sodium oxalate from nonevaporated spent liquor through seeding with over 50 g/l $Na_2C_2O_4$ seed.

Further alternative schemes have been tried on a large pilot scale, namely

- liquor calcination with added bauxite
- liquor ozonation
- precipitation of oxalate organic mixtures in fluidized bed columns
- precipitation of oxalate in packed spray trickle columns
- precipitation of oxalate from destabilized spent liquor
- precipitation of Bayer organics from deeply evaporated spent liquors.

The above listed processes have been considered during this study but may not yet be economically viable for large scale industrial operations.

(c) Biological Processes

Alcan has been considering the implementation of a biochemical process to remove the oxalates. Three possible uses for biochemical processes have been proposed.

Breakdown of the organic component in the evaporator cake which is discharged from the Bayer process.

Removal of organic carbon from bauxite before it enters the Bayer process streams.

Removal of organics from selected Bayer process streams.

Most biochemical processes operate at a pH close to 7, which makes it unlikely that a process will be found to operate under the very alkaline conditions of the Bayer process. Consequently, the second use described above would appear to offer the best prospect to

overcome this problem. A possible application would be to spray the mined bauxite ore in a stockpile with a solution containing the bacteria. Depending on the time required for the reaction to proceed, the spraying could be performed either at the mine site or at the process works. A flowsheet on this basis shown as Figure 5.1.

Kaiser Aluminum also encounters problems similar to those at Alcan. Discussions have been initiated with the US Bureau of Mines concerning studies in the field of humic acid removal.

Personnel at Dorr-Oliver, a major supplier of process equipment, have also expressed concern about the sodium oxalate crystallization, which causes filtration problems. Techniques such as spray burning of the spent liquor have been tested as a means of alleviating this problem. Other techniques, such as seeding the spent liquor with calcuim oxalate and washing the filter cakes to remove sodium oxalate have also been tested by Dorr-Oliver.

Obviously, this problem is a serious one which affects companies both within Canada and overseas.

5.2.2 - Uranium

In the uranium industry, the problems are confined to those ores of sedimentary nature.

(a) Background

The presence of humic substances in sedimentary sand, silt stone (roll front deposits) and carbonaceous deposits is the cause for detrimental effects in various unit operations for the extraction of metals.

In uranium extraction, the organic compounds in the ore are readily dissolved during leaching, becoming part of the pregnant solution. In the ion exchange stage of the process, the dissolved organic contaminants are poisoning the resin, thus decreasing the available reaction sites for uranium adsorption.

Humic acids and other organic substances in uranium ore deposits also have an adverse effect on the operation of solvent extraction circuits. The organic substances are extracted in the solvent extraction organic diluent and are one of the causes of emulsion and crud problems. The degradation products of humic substances form insoluble salts which cause the crud formation. One reason for this problem is believed to be a reaction between amines and humic acid. Low value pregnant strip solution could also be caused by an amine humic acid reaction.

(b) Conventional Processes

There has been limited work performed to understand the problems presented by uranium bearing ores. Several process alternatives which have been investigated are listed below.

Ore Blending

This method requires blending of the uncontaminated ore with the contaminated (humic matter) ore. The degree of blending will depend on the level of contaminant organics, type of equipment and process limitations. Some plants equipped with countercurrent decantation thickeners can absorb the tramp organic load more smoothly (without affecting the metallurgy) than those with filtration units.

Ore Roasting

This method requires roasting of the contaminated run-of-mine ore prior to milling. The roasting of the ore is very expensive in both capital and operating costs.

Adsorption with Activated Carbon

A granulated carbon can be added to the countercurrent decantation circuit to adsorb the tramp organics from the pulp stream. Alternatively, the pregnant liquor can be passed through columns packed with activated carbon. The spent carbon can be reused after regeneration. This method not only increases capital and operating costs but slightly reduces the uranium content of the pregnant liquor.

Oxidation of Organic Contaminants

An oxidizing agent can be added to the leach circuit to convert the hydrophobic organic contaminants into hydrophilic nonorganic soluble compounds. Economically, this is the low cost solution to the tramp organic problem in the pregnant liquor. However, extreme precautions must be exercised in the addition of an oxidizing agent. Adding excessive amounts of oxidant not only dissolve more silica during leaching but will also increase the degradation rate of Alamine 336 and isodecanol resulting in poor phase disengagement in the subsequent solvent extraction process.

Miscellaneous

The use of scrubbing agent containing

i) 5% NaOH or Na_2CO_3 and 5% methanol and

ii) tricelerators to remove dissolved carboxylic acids derived from tramps or humics in the feed has been suggested.

(c) Biological Processes

Two possible applications of biochemical processes to overcome the problems associated with the humic acids in the ion exchange and solvent extraction units are

removal of organic carbon from the uranium ore before it enters the uranium mill

removal of organics from the pregnant solution before it is fed to the ion exchange circuit. The rate of reaction of the biochemical process will have a major impact on the choice of the best alternative.

The field of resin poisoning and solvent extraction crud formation is of great concern to the uranium industry. Canadian consultants and equipment suppliers recognize the need for work in this area, even though the problem applies predominantly to uranium ores outside Canada.

5.2.3 - Other Industries

(a) Background - Flotation Circuit

Humic acids and other organic substances in water cause detrimental effects on the performance of some flotation circuits. This problem has been reported by Kidd Creek in the pyrrhotite flotation circuit, where the effect is seasonal, i.e. occurring during spring runoff season. Kennecott has also shown that humus organics adversely affects the flotation recovery of molybdenum. Also in column flotation studies, the humic acid content in process water has been identified as the cause for upset conditions.

(b) Biological Process

A possible use of a biochemical process to overcome this project would be to treat the process water before it is fed to the concentrator.

Note: This problem obviously affects companies both within Canada and overseas.

(c) Gold Processing

Mintek of South Africa have indicated that it has been investigating the effect of humic substances on the adsorption of gold on activated carbon for over a year and will shortly publish these results.

The humic materials were .found in tailings dumps containing vegetation and in outcrops associated with surface vegetation.

5.3 - Biological Destruction Methods

5.3.1 - Research Strategy

A significant problem encountered in the Canadian aluminum industry is due to the presence of humic and other substances in bauxite ores. During the Bayer process these substances undergo alkaline degradation, causing an accumulation of products which are responsible for downstream processing problems. As discussed earlier, biological methods do not appear compatible with the pH of the Bayer process liquor. To attempt to remove the contaminants such as oxalic acid, other aliphatic carboxylic acids and humic acids from the liquor by a biological route is not considered feasible. Thus, a method should be sought to prevent their presence in the Bayer liquor by removing the parent compounds from the ores prior to processing.

Based on the literature search reported earlier, there is no biological catalyst which can immediately serve as the basis of a process directed at solving this problem. In this section and subsequent sections, we will assess the application of a hypothetical, but desirable, process for the solution of the above problem. Key factors which must be addressed during the research and development of a biological method to remove the organic fraction from the bauxite ore will be identified. In addition to identifying those areas where significant information must be obtained, our examination of the conceptual process will highlight those factors which will influence the technical and economic feasibility of such a process.

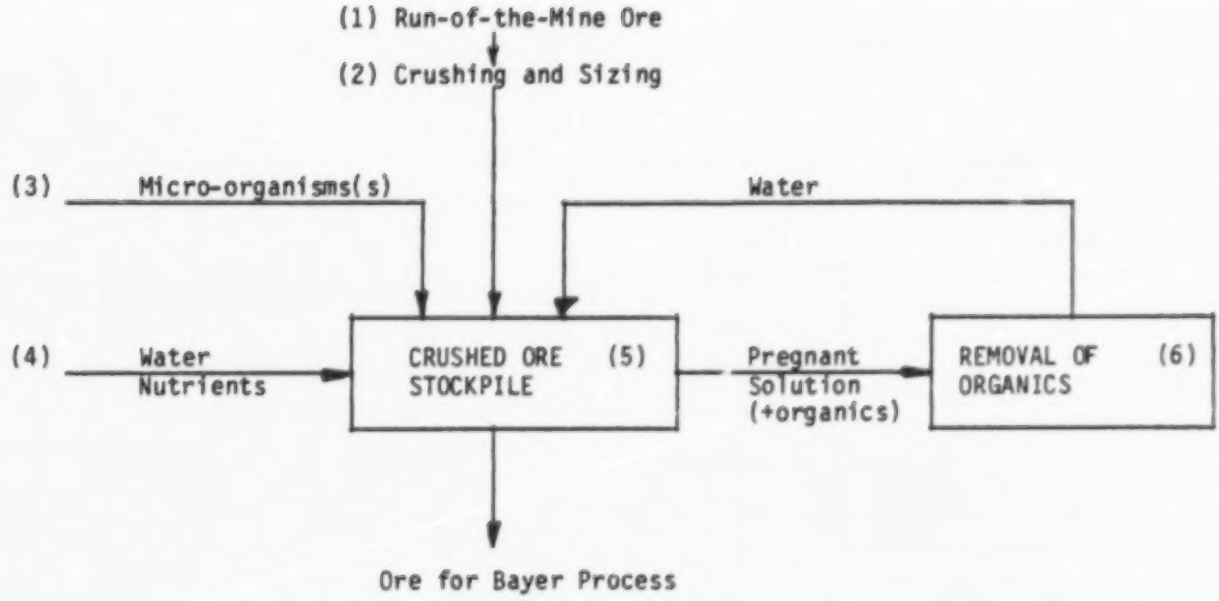

FIGURE 5.1 - HUMIC SUBSTANCES REMOVAL FROM ORES BY MICRO-ORGANISMS

5.3.2 - Hypothetical Process

This process is based on the removal of the parent organic compounds from bauxite ore prior to the Bayer process. The process flowsheet is shown in Figure 5.1. This process can be performed either at the mine site or at the refinery, the choice depending on the time required for the biological reaction to take place. Six unit processes or process streams are identified. Each of these reviewed below

(a) Run of Mine Ore

Since the goal is to remove those substances responsible for giving rise to oxalic acid, other aliphatic acids and humic acids in the Bayer process liquor, an important question still to be answered is which compounds have to be removed. In other words does oxalic acid, etc originate from all organic fractions present in the bauxite or do the contaminants only originate from the more readily soluble fraction of the humic materials (humic plus fulvic acids)? Alternatively, is kerogen a source of the contaminants?

In our literature search to date and in discussions with Alcan representatives, we know of no detailed information on this important aspect particularly as it applies to ores treated in Canada. The desirable information will include the precise characterization of the humic and other organic substances as they occur in an ore at any one site being investigated. It must also provide information as to the extent that this composition will vary within the ore.

In conjunction with these investigations, a study should be undertaken to identify the fraction(s) which is/are the source of the oxalic acid and other organic degradation products in the Bayer liquor. Biological processes are noted for their specificity, the above information will be helpful in identifying the target compounds in the crude ore.

This information is also important for another reason. If fractions such as humins or kerogens are identified as targets, the complexity of the task is expected to be greater due to those components being more refractory to biological attack than the lower molecular weight, less condensed humic and fulvic acids.

At the present time, it is not known to what extent the humins and kerogen compounds are degraded in the Bayer process. However, based on the chemical behavior of these components, it is suspected that the reactor conditions of the Bayer process will cause substantial degradation of these organics.

(b) Crushing and Sizing

Assuming that a micro-organism is being sought to actively degrade the humic/organic components, studies must be undertaken with the ore to ascertain the degree of particle size needed to expose the humics and organics versus additional cost of size reduction and materials handling. The permeability of the ore will also need to be investigated.

(c) Selection of Micro-organism

The final decision as to the type of organism being sought at this stage of the program will depend on the target compounds identified in the first unit process. During the literature search, the ability of selected bacteria, fungi and actinomycetes to degrade the lower molecular weight fractions of humins (humic acid or fulvic acid) was noted. Conversion factors as high as 80% were reported. As yet, no organisms have been identified as specifically decomposing humins and kerogens to a sufficient degree that they can be taken "off the shelf". However, it may be anticipated that such organisms do occur in nature but that a substantial screening program or enrichment culture approach will be needed to identify those organisms with the greatest ability to

degrade the organic materials of interest. This search may be facilitated if the organics can be isolated in sufficient quantity from the ore to allow their use as substrates in the microbial selection process.

As noted in the literature study, the ability of an individual organism to degrade humic fractions is both species and strain dependent. To find an efficient organism will be a major task. Nevertheless, a positive note is that compared with other topics of microbiology such as even the biological degradation of lignin, the microbial attack on humic materials is still a poorly understood process with room for considerable new development.

To achieve adequate rates and degree of degradation may require a mixed culture technique. This too will be a complex approach and care will be needed that organisms present do not cause recombination of low molecular weight phenolic degradation products with the reformation of melandoin (humus-like) pigments.

An important part of the process design will be the development of methods to prepare suitable inocula of the organism or organisms to be introduced into the ore stockpile. The generation of inocula of slow-growing organisms utilizing the humic substances as the sole carbon source may be a problem due to the lack of suitable substrates and the low growth rates. A possible solution to maintain an adequate microbial population will be to recycle organisms in the leachate. The use of alternative nutrient solutions may be feasible as long as the ability of the organisms to attack the organics in the ore is retained. However, such techniques may introduce substantial cost factors.

(d) Culture Conditions

Factors to be considered will include control of pH, temperature, moisture and level of aeration. The last-named factor is included

because, although all of the organisms noted in the literature are aerobic and require an adequate oxygen concentration for growth, excessive aeration can lead to the recondensation of phenolic type degradation products. The resultant dark melandoin polymers may be difficult to remove by leaching.

Although a heap leach technique would be preferred, a submerged or partially-submerged culture technique may be required to control oxygen levels and keep the organisms in contact with the organics for an adequate length of time.

The need for aeration may also impose a limit on the depth of a stockpile or necessitate some recycling and reaeration of the leach liquor.

Temperature will be influenced by the local environmental conditions and the optimum pH will reflect the requirements of the micro-organisms being used.

Nutrient requirements will depend on the respective organism(s) selected for the process. At the present time, none of the organisms identified as attacking humic or fulvic acids have been shown so far to be effective against more refractory components. Organisms able to utilize the humic materials as their sole source of carbon may only require nitrogen and phosphorus supplements. These types of organisms would be preferable in the process since, in order to utilize the humic materials, they would also be actively degrading them. Partial degradation of the humics to soluble fractions would enable them to be removed more readily in the effluent. Total degradation need not be essential. The use of a minimal level of added nutrients and the limitation that organisms in the culture must be able to utilize the humics as carbon source will also inhibit growth of contaminant organisms.

Contamination by other faster growing heterotrophes could be a potential problem if a supplementary carbon source is needed as well as the nitrogen and phosphorus. However, the humic materials may inhibit the growth of contaminant organisms.

As noted in the Stage 2 report, the attack of different bacteria, fungi and actinomycetes on humic fractions appears to be optimal when supplementary carbon and nitrogen compounds are present, i.e. the attack involves a secondary metabolic reaction. However, the presence of a readily utilizable carbon source would encourage the growth of other types of heterotrophic organisms and in view of the extended incubation periods expected to be required for degradation to be complete, the risk of contamination from the natural environment will be high.

Organisms able to utilize the humics and organics as the sole carbon or energy source would be preferrable for such a process.

(e) Culture Design

Any system designed for this process must take into account relatively long incubation periods. The isolation of organisms able to more effectively degrade the organics may allow these periods to be reduced. However, based on the refractory nature of much of the humic and kerogen fractions to biological degradation, this will be a difficult task.

The particulates of the ore will influence the rate of the process in two different ways. Soluble humic and fulvic fractions adsorb readily to clay particles and also possibly to other aluminosilicates in the ore. The presence of clay particles has been observed to decrease the rate of decomposition of fulvic and humic acids in liquid culture and a similar effect might be expected in the process. However, the chemisorption of the low

molecular weight organic fragments will also interfere with their removal by leaching from the stockpile. At the end of the treatment process, a chemical leaching with dilute alkali may be helpful in removing the adsorbed organics and possible melandoin pigments described in an earlier section.

Water requirements are expected to be high, while at the same time continuous removal of the degraded humic materials is also desirable to prevent reactions such as recondensation of the lower molecular weight phenolics. Recycling of the water/nutrient solution will be an economic benefit but will require that a purification procedure be developed to remove the trace concentrations of humic materials. Total destruction of the humic/organic fractions in the ore to carbon dioxide would have many benefits. However, it is more probable that the hydrolytic reactions will be the more effective approach to removing the humic materials. Removing the organics before the organisms will metabolize them will also encourage more hydrolytic activity on the part of the organisms as they seek an energy source.

The question of recondensation of low molecular weight components before they can be removed from the orepile will be an important factor. More information is needed on the effects of oxygen and nitrogen containing compounds on the possibility of recombination occurring. It may also be controlled by careful selection of the organisms used in the process.

6 - CONCLUSIONS AND RECOMMENDATIONS

6 - CONCLUSIONS AND RECOMMENDATIONS

6.1 - Conclusions

Problems caused by the presence of humic substances and other organic fractions, are encountered throughout the mining industry. The humic substances can either be present in the mined ore or be contained in the process water used during metals extraction and recovery. In general, little documentation on the phenomenom is available, but the problems appear to be of most significance in the aluminum, uranium and gold industries. The types of problems encountered in the uranium industry include the formation of cruds or the complexing of metals by humic substances during solvent extraction operations. In the case of the aluminum industry, the problems surface as accumulations of the degradation products of the humic and other organic materials in the ore in the process liquor of the Bayer process. These products have been characterized in part and include oxalic acid, other aliphatic carboxylic acids and humic-type residues.

Relative to the Canadian mining requirements, the processing of aluminum would benefit most from a solution to these humic-related problems.

Three alternative strategies for the use of biological methods to decompose or destruct the troublesome organic compounds in the aluminum processing circuit have been identified by Alcan. These include

(a) the biological degradation of the organic component in the evaporator cake discharged from the Bayer process

(b) biological destruction/leaching of the parent organic compounds as they occur in the bauxite ore prior to it entering the Bayer process circuit

(c) biological degradation of the organics from selected Bayer process streams.

The highly alkaline conditions within the Bayer process are not considered to be readily compatible with biological organisms that are able to degrade the contaminants such as oxalic acid. Methods requiring neutralization of liquors or cakes are expected to introduce unacceptable reagent cost factors. For this reason, the second alternative, i.e. the treatment of the parent organics before the ore enters the Bayer process, is viewed as the most attractive preliminary approach to a biological solution of the problem.

Humic materials and kerogen-type compounds are very resistant to biological attack. The vast majority of studies on the biological decomposition of humic materials has concentrated on the less condensed, lower molecular weight humic and fulvic acids. Conversion efficiencies of up to 80% have been recorded in the literature with selected humic fractions and organisms, but for the majority of micro-organisms investigated, a conversion efficiency of 40-55% would be rated as better than average. Most of the studies use decolorization or loss of absorbancy as an indicator of humic degradation, but it must be verified that fragmentation of the humic materials does occur.

Incubation periods required to achieve the stated conversion efficiencies are generally long and no information has been obtained from the literature regarding the rate of degradation of specific humin or kerogen fractions. Being more highly condensed than the humic and fulvic acids, it is probable that the levels of degradation will be lower and take longer to occur than in the case of the lower molecular weight fractions. Kerogens may be regarded to be of different composition than the humates although they have the common alkaline degradation product of oxalic acid. The rates and the extent of biological degradation of the kerogens will depend on their origin and composition. They are expected to be highly resistant to biological decay.

It is not known whether kerogen-type compounds have been identified in bauxite ores, or whether the degradation products identified in the Bayer process liquor are derived from all the organic compounts in the ore or only from a few of the more readily decomposed fractions. If the more highly condensed types of humin or kerogen materials are not significant sources of the oxalic and other aliphatic carboxylic acids, the task for a biological process would be potentially simpler. More detailed information on the composition of the organic fraction as it occurs in the bauxite ore would be an asset and would assist in the selection of a biological system for the specific removal of major sources of downstream contaminants.

A hypothetical process to achieve the biological degradation of humic and related organic compounds in bauxite ore prior to chemical processing has been described. Many questions remain unanswered with the current level of information. However, based on this concept, several key points are noted.

(a) Such a process is still at a very early stage of research and development.

(b) Organisms proven to degrade the humus or organic fractions in the bauxite ore have yet to be identified or isolated.

(c) The compounds that should be preferentially degraded are still not characterized.

(d) Organisms able to utilize humic materials as their sole carbon source would be preferable for a heap leach process open to the environment. The rate of decomposition by such organisms tends to be very low, but nitrogen and phosphorus supplements usually improve the growth of the organisms. More active degradation of humic or fulvic acids has been obtained in liquid culture containing both carbon, nitrogen and phosphorus supplements. If both carbon and

nitrogen supplements are added, the risk of contamination by other heterotrophic, faster growing organisms which are not humus degrading, may be high. In this respect, the presence of the phenolic-type degradation products of the humics may act as inhibitors of the growth of contaminant organisms.

(e) Degradation products of the humates may adsorb on to the surfaces of clay or other silicate fractions in the ore. To ensure adequate removal of the organics from the ore prior to processing, a chemical leach with dilute alkali may help to remove the chemisorbed organics.

6.2 - Recommendations

Based on the conclusions described above, a research program will be required to investigate the practical feasibility of this approach. Such a program might include the following stages

Phase 1 - Isolation of organic components of selected bauxite ores (both soluble and insoluble fractions) in consultation with the Canadian aluminum industry.

Phase 2 - Screening of organisms for their ability to degrade the fractions identified in Phase 1. This would include the more promising organisms identified in the literature search, i.e. bacteria, fungi and actinomycetes.

Phase 3 - Determine whether all fractions give rise to oxalic acid and other organic degradation product under the conditions of the Bayer process. Select the organism(s) best able to degrade the parent compounds as they occur in the ore (including the effect of added nutrients, etc).

Phase 4 - Undertake bench-scale tests to evaluate the ability of the candidate organisms to degrade the humates in a bauxite ore sample.

Following these sets of tests, a decision could be made as to whether more detailed bench tests leading to a pilot-scale would be justified.